



HANDBOOK OF  
MEDICAL PROTOZOOLOGY

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# HANDBOOK OF MEDICAL PROTOZOOLOGY

*For Medical Men, Parasitologists  
and Zoologists*

*by*

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*To*  
CLIFFORD DOBELL  
*My Guide, Philosopher and Friend*

“Infection must be considered as a struggle between two organisms, the parasite and its host. . . . This struggle brings about adaptations on both sides.”

METCHNIKOFF, 1891.

## PREFACE

AMONG the infectious diseases affecting mankind those caused by protozoa, though not so numerous and widespread as bacterial and virus diseases, are equally important, especially in the tropics and other warm countries, where Malaria, Amœbic Dysentery, Kala-Azar and Sleeping Sickness—to name the most serious protozoal infections—have a profound influence upon human health and general welfare. In view of the prevalence of protozoal diseases in tropical and subtropical regions, the study of the protozoa and the diseases caused by them is divorced from the general medical curriculum and assigned to the domain of tropical medicine. As a result of this specialization, the medical student and the general practitioner have only a superficial acquaintance with the protozoa parasitic in man, based on second-hand information, usually acquired from textbooks of bacteriology or pathology, to which these organisms have been relegated. Since in most cases the authors themselves are not familiar with the subject, the account of the protozoa given in some of these books is full of mis-statements and anachronisms, and therefore not only inadequate but frequently misleading.

The segregation of tropical medicine from general medical training is, however, purely artificial, for the distribution of some protozoal diseases actually extends far beyond the tropical zone into countries with a temperate climate. Furthermore, the barriers which formerly prevented the spread of these infections beyond their natural boundaries have been broken down by modern means of rapid transport, especially by air, while opportunities for the introduction of individual cases of protozoal infections into this country have multiplied considerably as the result of the recent war. Under these conditions the general practitioner in this country, as well as in other temperate regions, is liable to encounter cases of protozoal diseases in increasing numbers.



A sound knowledge of the human protozoal infections is thus essential not only for medical officers in the tropics (Colonial Service, missionary and industrial fields, the Forces), but also for the medical practitioner at home. But while members of the first group usually receive adequate instruction in medical protozoology in postgraduate courses of tropical medicine, provision for the study of this subject available to the undergraduate student and the general practitioner is practically non-existent.

The present handbook, which was written in an endeavour to satisfy an existing demand for a short textbook devoted exclusively to medical protozoology, is a greatly expanded version of lectures delivered by me during the recent war to postgraduate students studying for the Diploma of Tropical Medicine and Hygiene and to medical officers of the Allied Forces taking short courses in tropical diseases and parasitology. This book is primarily intended to fill a gap in the medical curriculum by providing students and practitioners of medicine with all the essential information regarding the protozoa living in man and the diseases caused by them. It is also adapted to the needs of colonial medical officers, both as an aid in their studies for the diploma and as a guide in their practice overseas. It is hoped that it will also be of service to clinical pathologists, parasitologists and laboratory workers in general, as well as to veterinary practitioners. To the latter the interest lies chiefly in the comparative aspects of medical protozoology, in view of the part played by lower mammals as reservoir hosts of human protozoal infections. Lastly, this book should be of interest to zoologists, by giving a closer insight into the protozoal fauna of man and the host-parasite relations between them, a subject which receives scant attention in general zoology.

I have restricted the presentation of this subject to essential facts concerning the protozoa in man, without mentioning the historical development of our knowledge and omitting all reference to authorities. The treatment is thus frankly didactic and somewhat dogmatic, which is unavoidable if the student is not to be confused with conflicting data and conceptions which he is not in a position to verify himself. Special emphasis is given to the host-parasite relations between man and his protozoal parasites, but the purely clinical aspects of protozoal diseases are dealt with only in so far as they reflect the interaction between parasite and host, while the

therapeutic treatment of diseases is omitted as being outside the scope of this work. On the practical side, a selection has been made of those methods of parasitological diagnosis which, in my experience, are the most reliable and simple to use. The systematic description of the human protozoa is preceded by an introductory section, dealing with the general principles of protozoology and the fundamental conceptions of parasitology, which provide the necessary theoretical background to the subject. The reader is also introduced to the Rules of Zoological Nomenclature, which form the basis of the scientific classification of the animal kingdom.

For fuller information on the clinical and therapeutic aspects of protozoal diseases the student is directed to textbooks and monographs of tropical medicine, a list of which will be found at the end of the book. For the benefit of those who desire to learn more of the subject, classified lists are also given of publications in different branches of protozoology.

In writing this book I have consulted the works of numerous authors, incorporating those views with which I find myself in agreement. Though unnamed, these sources of information are fully appreciated. I have benefited considerably from discussions with my friends and colleagues, and desire to record my gratitude to the late Dr. C. M. Wenyon, F.R.S., Dr. Clifford Dobell, F.R.S., Dr. D. J. Bauer, Dr. J. C. Broom, Mr. L. G. Goodwin and Dr. C. J. Hackett. I also wish to express my deep appreciation of the generous assistance received from Miss I. Bellis, to whose patient and critical perusal of the manuscript many improvements of the text are due.

The illustrations include many *original figures*, which I have specially drawn for this book. Some of the composite figures and coloured plates have been designed to serve as keys for the differential diagnosis of the more important groups of parasitic protozoa. Other figures have been adapted or reproduced from various sources, which are acknowledged in the accompanying legends. I am indebted to the authors and publishers named for permission to use these figures. I am also grateful to my colleague, Mr. B. Jobling, for the artistic execution of the coloured plates.

All the measurements given in this book are based on the International System of Weights and Measures, commonly known as the metric system. Since the recognized unit of volume, the

litre, has no connexion with the metre, the thousandth and millionth parts of a litre are designated as millilitre (ml.) and microlitre ( $\mu$ l.) respectively, and not as cubic centimetre (c.c.) and cubic millimetre (c.mm.). Though incorrect, the last two units are unfortunately still in use, especially in medical publications.

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# **PART I**

## **GENERAL ACCOUNT OF THE PROTOZOA**

### **CHAPTER 1**

#### **INTRODUCTION**

PROTOZOOLOGY is a branch of general zoology devoted to the study of the group of microorganisms of animal nature which are known as Protozoa. Among the protozoa there are about thirty species which are parasitic in man, some of them causing diseases, while others are more or less harmless. The study of the human parasitic protozoa in general and of the pathogenic protozoa in particular forms the subject matter of medical protozoology. Though primarily concerned with the protozoa living in man, medical protozoology also includes certain protozoa which occur in lower mammals but have a direct bearing on human protozoal infections, because some of them are parasites common to man and other mammals, while others, though distinct, occur in animal hosts in which human protozoa may also be found.

In dealing with protozoal diseases it is necessary, first, to detect the protozoa in the patient ; secondly, having found them, to recognize or identify the pathogenic forms and to differentiate them from harmless protozoa occurring in man ; and, thirdly, to combat or treat the diseases caused by these protozoa. We are concerned here only with the first two problems, and this book is accordingly devoted (a) to a systematic description of the structure, life-cycles and bionomics of the parasitic protozoa ; (b) to the mutual relations of the parasites and the human organism (host-parasite relationship), and (c) to the methods employed in the parasitological diagnosis of protozoal infections.

#### **STATUS OF THE PROTOZOA**

The morphological or structural unit of all living organisms is represented by the CELL, which is typically composed of a mass of protoplasm consisting of nuclear substance (chromatin) and cytoplasm. The type of cellular organization serves as a basis for



the classification of the Animal Kingdom into two main groups or Subkingdoms: the METAZOA and the PROTOZOA.

The METAZOA comprise animals, the bodies of which are built up of numerous cells which are differentiated to form various tissues and organs, each adapted to perform its particular physiological function. The Metazoa are thus *multicellular animals* in which there is a division of labour between diverse types of cells.

The PROTOZOA are animals of microscopic dimensions, the body of which is composed of a single morphological unit corresponding to a metazoal cell, on account of which they are designated

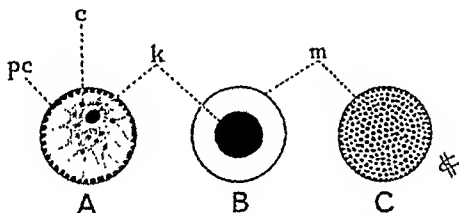


FIG. 1.—NUCLEI OF PROTOZOA (Original).

A. Vesicular nucleus (*Entamoeba coli*) with (pc) chromatin granules (peripheral chromatin) on the nuclear membrane (m) and (c) between this and the karyosome (k); B. Vesicular nucleus with chromatin restricted to the karyosome (k); C. Massive nucleus.

*unicellular animals*. However, in contrast to the metazoal cells, which are differentiated according to their special functions and are mutually dependent on one another, the protozoon may reach a high degree of morphological and physiological complexity, since it is adapted to perform all the vital functions within the scope of a single cell. Therefore, each protozoon is in every respect a complete and independent organism, equivalent to the entire multicellular animal and not to one of its constituent cells. On account of this and because the body of a protozoon is not partitioned into cells, some authors refer to the protozoa as *non-cellular* organisms, to distinguish them from the metazoa, which are described as *cellular* organisms. However, the distinction between the two groups is not very sharp, for there is every reason to believe that the Metazoa have evolved from the Protozoa. This evolution

is indicated, in the first place, by the embryonic development of the Metazoa, in the course of which they pass from a unicellular condition, represented by the fertilized ovum, to a multicellular condition; and, secondly, by the existence among the Protozoa of colonial forms. These show every gradation from a loose assembly of undifferentiated individuals, or zooids, enclosed in a common gelatinous matrix, to colonies, in which the zooids are differentiated into vegetative, or somatic, cells and reproductive cells, connected with each other by protoplasmic bonds. There is thus a transition from unicellular to multicellular animals.

Though the protozoa are usually assigned to the Animal Kingdom, this view cannot be accepted unreservedly. There is no difficulty in distinguishing between the higher multicellular plants and



FIG. 2.—NUCLEAR STRUCTURE OF BACTERIA AND SPIROCHAETES.

(Adapted from Robinsow, 1944, and Dobell, 1911.)

Chromatinic elements (chromosomes) scattered in the cytoplasm: a-c. Bacteria; f. Spirochaete.

animals, the Metaphyta and the Metazoa, which differ radically in their physiological activities. Typical plants take up as food simple compounds of carbon and nitrogen, in the form of carbon dioxide from the air and ammonia from the soil. From these they build up, on the one hand, carbohydrates; on the other hand, proteins. Animals, however, are unable to assimilate the simple compounds but must obtain their food ready made from plants or other animals. Hence animals depend for their food on other living beings. Thus, the function of plants is perpetual synthesis, while that of animals is analysis. This difference extends to the lower or unicellular organisms, some of which—like the lower algæ, micro-fungi, yeasts, bacteria and spirochaetes—are assigned to the Vegetable Kingdom, and others—like protozoa—to the Animal Kingdom.

Though many of the protozoa conform to the conception of

animals, there are others, especially among the free-living forms, which are either definitely plant-like or show a mixture of plant and animal features. Such forms are claimed both by botanists and by zoologists. To avoid this confusion it has been proposed to unite *all* unicellular organisms in an independent group or Kingdom, the PROTISTA, and to subdivide them into two main groups, according to the type of nuclear structure. Members of the first group, the Prokaryota, represented by the bacteria and the spirochaetes, do not possess a true nucleus but the chromatin-substance is scattered in the cytoplasm in the form of chromosomes (Fig. 2). Members of the second group, the Eukaryota, comprising the protozoa, unicellular algæ and micro-fungi, have a distinct nucleus (Fig. 1), separated from the cytoplasm by a membrane, within which all the chromatin-substance is concentrated. As stated already, the Protozoa comprise both plant-like and animal-like forms, which will be dealt with more fully below.

### Structure of Metazoal Cell

Since the body of a protozoon exhibits the same fundamental features as the cell of a metazoon, it will be expedient, for comparison, to recall the typical structure and methods of reproduction of the cell in higher animals before turning to the morphology of the protozoa.

In the Metazoa the cell is composed of protoplasm, which consists of two substances, the nucleus and the cytoplasm, together with various cytoplasmic inclusions (Fig. 3, A). The nucleus is separated from the cytoplasm by a nuclear membrane (Fig. 3, A, m). Within the nucleus there is a network, or reticulum (Fig. 3, A, r), which is made up of thread-like chromosomes containing a darkly staining substance, the chromatin. However, in the non-dividing or resting nucleus the chromosomes cannot be distinguished individually. The chromatin is usually combined with faintly staining substances described as achromatin. In addition to these structures, the nucleus usually contains a rounded body, the nucleolus (Fig. 3, A, n). All these elements are embedded in a fluid, the nuclear sap. The nuclear constituents are selectively coloured by certain stains and can be identified by their staining reactions as well as by other chemical tests. The chief functions of the nucleus are connected with assimilation of food (metabolism) and with

reproduction, the chromosomes playing the most important part in the latter process. Another important inclusion of the cell is the centrosome (Fig. 3, A, cc), a granule which lies outside the nucleus and is concerned with nuclear division.

### Division of Metazoal Cell

The most important process in the reproduction of all cells is the nuclear division, which in most cells of the Metazoa takes place by the indirect method or MITOSIS (= *karyokinesis*), in the course of which the nucleus passes through four successive stages: prophase, metaphase, anaphase and telophase.

In PROPHASE (Fig. 3, B, C), the chromosomes, which formed a tangled network and were indistinguishable in the resting nucleus (Fig. 3, A, r), are resolved into separate thread-like structures. The number of chromosomes in the cells of each animal species is constant (e.g. in man there are 48 chromosomes) and the appearance of the individual chromosomes varies but there are always present two of each kind, known as the *diploid* set (Fig. 3, B, c). Later in the course of prophase each chromosome splits lengthwise and is thus doubled but the two parts remain attached to each other (Fig. 3, C, dc). In the meantime the centrosome lying outside the nucleus divides into two (Fig. 3, C, a). By the end of the prophase the nucleolus and nuclear membrane disappear and the process of division enters into the metaphase. In METAPHASE (Fig. 3, D) the two centrosomes or asters (a) migrate to the opposite poles of the cell, while between them is formed a *spindle* (s), which consists of gelatinous strands. The paired chromosomes then become arranged in the broadest part of the spindle to form an *equatorial plate* (Fig. 3, D, eq). In ANAPHASE (Fig. 3, E) the halves of each double chromosome separate and move to the opposite poles. Finally, in TELOPHASE (Fig. 3, F) the spindle disappears and each daughter-group of chromosomes becomes surrounded by a new nuclear membrane. At the same time the cytoplasm becomes constricted in the middle, the cell divides into two (Fig. 3, G), and the nucleus of each daughter-cell is reconstructed, assuming the structure seen in the resting stage. As the result of mitotic division each daughter-nucleus receives a set of chromosomes similar in number to that of the parent-cell and characteristic of the species of animal to which the cell belongs. Mitosis thus ensures an equal distribution in

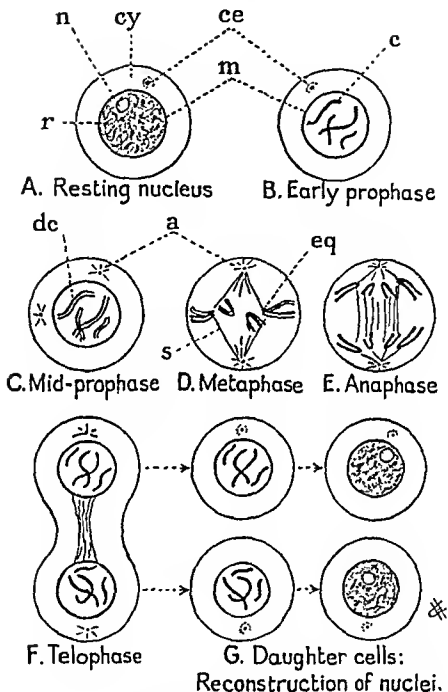


FIG. 3.—DIVISION OF NUCLEUS BY MITOSIS (*Original*).

a. Asters; c. Chromosomes; ce. Centrosome; cy. Cytoplasm of cell; dc. Double chromosomes; eq. Equatorial plate; m. Nuclear membrane; n. Nucleolus; r. Nuclear reticulum; s. Spindle.

the daughter-cells of the hereditary elements borne by the chromosomes.

Another method of nuclear division, which rarely occurs in

animal cells, is the direct method, or AMITOSIS, in the course of which the nucleus is simply constricted into two halves, each of which gives rise to a nucleus in a daughter-cell.

While the nucleus of vegetative or somatic cells of an animal is characterized by a diploid set of chromosomes, in mature sex cells the number of chromosomes is halved. This is brought about by two nuclear divisions preceding the formation of the gametes, which are known as reduction divisions or MEIOSIS. The general course of the first nuclear division is similar to that in mitosis and results in the formation of two diploid daughter-nuclei, but it involves an interchange of parts of the chromosomes, leading to a redistribution of paternal and maternal elements. However, in the second nuclear division the chromosomes do not split into two but are sorted out equally between the daughter-nuclei, each of which thus receives half the number of somatic chromosomes which is known as a *haploid* set. When the sex cells unite during fertilization, the haploid sets of chromosomes of the male and female gametes fuse, thereby restoring the diploid number of chromosomes in the nucleus of the fertilized ovum, or zygote. The diploid chromosome number is thenceforth maintained in the somatic cells of the organism by mitotic divisions.

### STRUCTURE OF THE PROTOZOA

The great majority of protozoa are of microscopic size, which is measured in microns ( $1 \mu = 0.001 \text{ mm.}$ ). As in the case of a metazoal cell, the body of a protozoon is made up of cytoplasm and at least one nucleus, its structure differing widely in various types of protozoa and frequently reaching a high degree of complexity.

The cytoplasm consists of a viscous substance which may contain various inclusions and organs adapted to perform different vital functions, such as protective, locomotor, metabolic and sensory. The cytoplasm is usually differentiated into a hyaline outer layer, or ECTOPLASM, and a granular or vacuolated inner mass, or ENDOPLASM. The ectoplasm, being protective in function, is more compact than the endoplasm, the degree of its rigidity determining the shape of the body. Its surface may be modified into a denser layer, or periplast (e.g. in amœbæ), or into a firm membrane, or pellicle (e.g. in ciliates). It may also secrete a tough membrane, or cyst, which protects the protozoon against external influences, while

some free-living protozoa (e.g. Foraminifera) are enclosed in a permanent hard shell, which is also produced by the ectoplasm. Other protozoa (e.g. flagellates and ciliates) are provided with supporting skeletal elements. The ectoplasm also gives rise to the organs of locomotion and accessory structures, which are described below. The endoplasm is mainly concerned with nutrition and reproduction, and contains various inclusions and organs, or organellæ, the most important of which is the nucleus. The inclusions are represented by food-vacuoles, stored food substances and other structures.

The nucleus of protozoa consists essentially of the same elements as that of metazoal cells but their structure and arrangement are more varied than in the latter. There are two main types of protozoal nuclei: (a) the *vesicular* and (b) the *massive*. In the vesicular type (Fig. 1, A, B) most of the nuclear space is free of chromatin, which may be either situated on the inner surface of the nuclear membrane or embedded in a special rounded body, known as the KARYOSOME (Fig. 1, B), or in both these positions (Fig. 1, A). The chromatin granules lining the nuclear membrane constitute the so-called PERIPHERAL CHROMATIN. Among the protozoa living in man the nucleus is most commonly of the vesicular type. In the massive type the nucleus is more or less evenly filled with chromatin granules (Fig. 1, C).

**Organs of Locomotion.**—Locomotion in protozoa is the function of the ectoplasm, which may be differentiated in various ways to produce special organs, or organellæ, of locomotion, of which there are three main types: PSEUDOPODIA, FLAGELLA and CILIA. In some forms, like amœbæ, movement is effected by extrusion of cytoplasmic outgrowths, known as pseudopodia (Fig. 5, b), which arise from any part of the body and are retracted when the organism ceases to move. Progression of this type, known as *amœboid movement*, is also observed in malaria parasites. Other protozoa, known as flagellates, move by means of flagella (Fig. 19, d, e), representing filamentar or whip-like processes, the lashing movements of which propel the organism. In the infusoria, or ciliates, the body is covered with numerous short hair-like processes called cilia (Fig. 18, f, g, h), which vibrate in unison and cause the organism to progress. While the pseudopodia of amœbæ are temporary structures, without a definite localization in the body, formed during locomotion and

withdrawn when the organism is at rest, both the flagella and cilia are permanent organellæ, though, under certain conditions, they may temporarily disappear. The flagella and cilia have essentially the same structure. Each of these organellæ arises in the cytoplasm from a minute dark-staining body, known as the **BASAL GRANULE**, or **BLEPHAROPLAST** (Fig. 19, c), and consists of an elastic axial filament, or **axoneme**, enclosed in a protoplasmic sheath which is continuous with the ectoplasm. In addition to the simplest forms of locomotor organellæ just described, others have become specialized in different ways. Thus in some flagellates a flagellum is attached to the outer margin of a frilled extension of the ectoplasm, the two forming an **UNDULATING MEMBRANE** (Fig. 19, f; Fig. 13, h-j) which serves for locomotion. In some ciliates groups of cilia have fused, giving rise to bristle-like processes (*cirri*) or membranes (Fig. 18, f-h), the movements of which help to drive the food into the mouth. The speed of locomotion in protozoa varies considerably. Thus, amœbæ move at the rate of from 0.2 to 3  $\mu$  per second, flagellates from 15 to 30  $\mu$ , and ciliates from 400 to 2,000  $\mu$ .

Some protozoa possess no special organs of locomotion but progress by slug-like or *gliding* movements (e.g. gregarines and young forms of coccidia) or by worm-like contractions of the body. Movements of the latter type, known as *metabolic*, enable the organisms to squeeze themselves between particles obstructing their path or, in the case of parasitic forms, to penetrate into various cells and tissues of the host.

### REPRODUCTION OF THE PROTOZOA

As in the case of metazoal cells, reproduction in protozoa is initiated by nuclear division, which is followed by fission of the cytoplasm and the separation of the daughter-individuals.

**Division of the Nucleus.**—In the majority of protozoa the nucleus divides by some form of mitosis, while amitosis is restricted to the macronucleus of ciliates. Among the varieties of mitotic division occurring in protozoa the following may be noted: (a) **PROMITOSIS**, representing a primitive form of mitosis, in the course of which the karyosome is drawn out in the form of a dumb-bell with a constriction in the middle, finally producing two so-called "pole-caps" at the extremities, while the chromatin granules lining the nuclear membrane (peripheral chromatin) give rise to the chromosomes,



which form an equatorial plate. These chromosomes split into two groups which move to the opposite poles and—together with the “pole-caps”—give rise to two daughter-nuclei. In this type of mitosis the nuclear membrane is retained throughout the division, which is therefore *intranuclear*. (b) *MESOMITOSIS* differs from typical mitosis only in the fact that the whole process of division takes place within the nuclear membrane (intranuclear). Finally, in (c) typical *MITOSIS* the membrane disappears early in the course of division and the process is indistinguishable from mitotic division in higher animals.

Whereas in many protozoa distinct chromosomes have not yet been recorded, in others typical chromosomes of definite shape and number make their appearance during nuclear division. The rôle of the karyosome in nuclear division varies. In some protozoa it gives rise both to the centrosome and the chromosomes, in others it acts as a centrosome itself, while in others again it provides only the chromosomes; in some cases, however, the karyosome takes no part in nuclear division but disappears.

**Division of the Body.**—Nuclear division in protozoa is followed by fission of the body, giving rise to two or more daughter-individuals, according to the type of multiplication. In the simplest case fission is *binary* (Fig. 5, c, e), when the body divides into two daughter-individuals of equal or unequal size which separate immediately and begin to lead an independent existence. In most protozoa the body divides longitudinally (e.g. in flagellates: Fig. 20) but in some it divides transversally (e.g. in ciliates: Fig. 16, c, d). In certain flagellates (e.g. the rat-trypanosome: *Trypanosoma lewisi*) the body undergoes a number of successive divisions without complete fission of the cytoplasm, with the result that several daughter-individuals in various stages of division remain attached to each other before breaking away. This type of multiplication is known as *multiple fission*. Another method of multiplication, known as *schizogony*, represents a form of multiple fission proceeding by stages. First the body becomes multinucleate by a series of rapid mitotic divisions of the nucleus; then each daughter-nucleus becomes surrounded by a bud-like portion of cytoplasm; and finally each bud becomes detached by constriction and the body undergoes fission, or segmentation, giving rise to young forms, or merozoites, the number of which corresponds to the number of nuclei present in the dividing animal, known as the schizont. In

this process a portion of unused cytoplasm (so-called residual body) is frequently left behind. Reproduction by schizogony is characteristic of the coccidia (Fig. 14, a-f) and the malaria parasites (Fig. 32, a-e; Fig. 33).

**Sexual Process.**—In some protozoa reproduction is exclusively asexual throughout their entire life-cycle, in the course of which the organism, which is known as a **TROPHOZOITE**, feeds and grows, then divides by one of the methods described, giving rise to daughter-individuals, in which the process is repeated. In other protozoa reproduction is accompanied by a sexual process, or **SYNGAMY**, which is of universal occurrence among the higher animals and plants.

In multicellular organisms the body is differentiated into the ordinary or somatic cells, on the one hand, and into sexual or germ cells, on the other hand. The mature sexual cells, or **GAMETES**, are represented by male forms, or spermatozoa, which are minute active forms, and by female forms, or ova, which are more bulky and inactive. After the gametes have been produced in the sexual glands (testis and ovary of the male and female respectively), they are released and a male gamete unites with a female gamete, their nuclei and bodies fusing into a single cell, the fertilized ovum, or **ZYGOTE**, which proceeds to multiply by division, ultimately giving rise to a new multicellular organism.

In the protozoa the sexual process differs from that in the metazoa in several points. Owing to the unicellular constitution of the protozoa, the gametes are represented by complete organisms which may or may not differ from the ordinary trophozoites and which are sometimes produced at certain stages of their life-cycle. In some cases there is no visible differentiation of male and female gametes, the structure of which may be similar (*isogametes*). In other cases the gametes may differ markedly (*anisogametes*), the males being small active forms (**MICROGAMETES**) with scanty cytoplasm, while the females are large inert forms (**MACROGAMETES**) containing reserve food-material. The stages preceding and giving rise to the gametes, which are known as **GAMETOCYTES**, sometimes also show sexual differentiation. The sexual union, or syngamy, may be effected by complete fusion of the two sexual individuals and is then known as **COPULATION**, or the two partners merely come in contact and exchange nuclear material, when the process is called **CONUGATION**. Copulation of anisogametes in coccidia (Fig. 14, k, o)

and in malaria parasites (Fig. 32, h, i), and conjugation, which is characteristic of the ciliates, occur among the protozoa living in man and are more fully described in Part II. As in the case of higher animals, the essential purpose of syngamy is the intermingling of the chromatin elements, or chromosomes, derived in equal parts from each of the two individuals participating in the union. In order to ensure that the zygote—and subsequently the trophozoite—contains the normal diploid number of chromosomes, the gametocytes undergo reduction divisions, or meiosis (see above), resulting in a halving of the number of chromosomes in the gametes, which have haploid sets. The diploid set of chromosomes is restored in the zygote after fertilization, by union of the chromosomes of the male and female gametes.

**Development.**—In the course of their multiplication the protozoa usually pass through a series of morphological changes, or stages of development, which together constitute their life-cycle. In some species the development consists merely of growth and reproduction of the organisms, which always appear under a similar form, differing only in size, and are therefore monomorphic, as in the case of *Trichomonad* flagellates (Fig. 13, h-j) and ciliates (Fig. 16). Other species assume a variety of forms at different stages of their life-cycle or in response to environmental changes. In the case of such polymorphic protozoa a knowledge of the whole life-cycle is required before any given form can be referred to its proper systematic position. Thus, trypanosomes reproducing asexually by binary fission pass through various stages in the course of their life-cycle (Figs. 25, 28), while in protozoa reproducing by schizogony there is considerable difference in size and appearance between the trophozoites, schizonts and merozoites, as in the case of coccidia (Fig. 14, a-f) and malaria parasites (Fig. 32, a-e).

Further complications are introduced by the occurrence of syngamy in the life-cycle. The sexual forms (gametocytes and gametes) may differ markedly in morphology from the asexual forms, on the one hand; and there may be a distinction between the male and female forms, on the other hand (Fig. 14, h, k, n, o; Fig. 32, g, h). In some protozoa (e.g. ciliates) syngamy occurs at irregular intervals in the course of their life-history, while in others (e.g. malaria parasites) it forms a definite part of the life-cycle, in which there is an alternation of asexual and sexual generations.

This type of development is characteristic of the Sporozoa, in which the sexual part of the life-cycle terminates in the production of forms that serve for the propagation of the parasites to new hosts. In some of these (e.g. the coccidia : Fig. 14) both the asexual and sexual development proceed in the same host, whereas in others (e.g. the malaria parasites : Fig. 32) the life-cycle is divided between two hosts, in one of which, represented by a vertebrate, the asexual development takes place, while in another, represented by a blood-sucking insect, the sexual development takes place. In such cases *alternation of generations* is combined with an *alternation of hosts*. The life-cycles of parasitic protozoa will be dealt with more fully in Part II. In addition to polymorphism associated with stages of development, some protozoa may assume different forms under the influence of external conditions, as in the case of *Dimastigamöba*, which possesses amöboid and flagellate stages (Fig. 18, a, b).

**Cysts.**—The majority of protozoa are adapted to withstand the influence of unfavourable conditions by forming resting stages, when the organism becomes invested with some form of resistant membrane. Such stages are collectively known as **CYSTS**. The function of cysts is in general protective but it may also be associated with multiplication and with propagation, in various combinations. The simplest case is observed in free-living protozoa. Under unfavourable conditions, such as exhaustion of the food supply, fouling or drying up of the medium, etc., the organism becomes rounded, loses some of its characteristic structures (e.g. flagella, cilia, cytostome) and secretes a resistant membrane or cyst-wall around the body. In the encysted state the protozoa can be dried up and remain viable for long periods, sometimes for months or even years. Cysts of free-living forms are found attached to plants, in the soil or at the bottom of dried water collections, whence they may be disseminated in various ways, e.g. by other animals or by the wind. When they are thus transported to a suitable medium or when conditions on the spot improve, excystation takes place, the organism escaping from the cyst and resuming its active existence. In some free-living protozoa encystment is combined with multiplication, thus some ciliates regularly multiply by division within a cyst, which then ruptures, releasing the daughter-individuals. After these attain full size, the process is repeated. The function of cysts in such cases is both protective and multiplicative.

While the majority of free-living protozoa encyst sporadically, as a reaction against adverse conditions of existence, in parasitic forms the production of cysts occurs more or less regularly in the course of their normal life-cycle and is an adaptation enabling the parasite to survive temporary exposure to external conditions after it escapes from the body of its host and until it is taken up by a new host, in which excystation takes place. Since in some parasitic protozoa encystment represents the only means of ensuring transmission from host to host, the function of cysts in such cases is propagative as well as protective, and may also be associated with multiplication.

Both in free-living and in parasitic protozoa the cysts vary considerably in appearance and structure. In some the wall consists of a single layer and its shape may be round (Fig. 4, c-f), oval (Fig. 13, m, n) or pear-shaped (Fig. 13, b). In others the wall may consist of more than one layer and secondary cysts may be produced within the primary one. Thus the resistant stage of coccidia is represented by a so-called oocyst, which encloses a number of secondary cysts, or sporocysts, within each of which several sporozoites are formed (Fig. 15, c, d, f). A special type of cyst occurs in the Cnidosporidia. The cysts, known as spores, of these parasites are provided with so-called polar capsules containing a coiled-up filament. Within the body of a new host these filaments are rapidly extruded, anchoring the spores to the wall. The cysts of different species of protozoa possess distinctive features, which, in the case of parasitic forms, provide important criteria for their identification and classification. Cyst-formation among the protozoa living in man will be dealt with more fully in Part II.

### PHYSIOLOGY OF THE PROTOZOA

As in other living organisms, the vital activities of the protozoa involve a discharge of energy, which is produced by the breakdown of foodstuffs. Their most essential physiological functions are accordingly represented by nutrition and by respiration.

Nutrition.—The food requirements and the methods of nutrition differ widely in various groups of protozoa. Some, like plants, are capable of building up foodstuffs from inorganic compounds, while others, like animals, depend upon pre-formed complex organic substances.

The purely plant-like or *holophytic* type of nutrition, which is also called *autotrophic*, is peculiar to protozoa (chiefly flagellates) possessing organelles (so-called plastids) bearing chlorophyll or other pigments (chromatophores), with the aid of which they utilize the energy of the sunlight to synthesize simple carbohydrates from carbon dioxide and water. These substances may be further elaborated to form complex carbohydrates, fats, and, together with nitrogen derived from ammonium or other inorganic compounds, proteins. This type of nutrition is, therefore, characterized by photosynthesis.

The animal-like or *holozoic* type of nutrition, which is also known as *heterotrophic*, is characteristic of protozoa devoid of chlorophyll and requiring an organic source of carbon. This is provided either by products of bacterial decomposition of organic matter found in solution, or by solid food consisting of other organisms or of formed particles. Holozoic protozoa are, therefore, dependent on other living organisms (animals or plants) as sources of food.

In the case of protozoa living on organic food material in solution nutrition is said to be *saprozoic*, the protozoa absorbing nourishment through the surface of the body into the cytoplasm, where the food is assimilated. Saprozoic nutrition is common among various free-living protozoa found in habitats containing decomposing organic matter and among parasitic protozoa. Sometimes it is combined with photosynthesis, thus representing a transition from the holophytic to the holozoic types of nutrition, known as *mixotrophic* nutrition. This type of nutrition is found in some flagellates (e.g. *Euglena*) which photosynthesize when exposed to light but feed saprozoically in the dark. In protozoa feeding on particulate matter the mode of nutrition is said to be *phagotrophic*, ingestion being effected either by pseudopodia, which actively engulf the food-particles (e.g. in amœbæ: Fig. 5, a, b), or through a special mouth-opening, the **CYTOSTOME**. The cytostome may be represented by a simple slit in the body-wall (as in some flagellates: Fig. 13, a, i) or it is situated within a depression of the body, the **PERISTOME**, which may be highly differentiated and is sometimes accompanied by a gullet or **CYTOPHARYNX** (as in some ciliates: Fig. 16, a). In some cases special flagella or cilia, as well as modifications of the latter (membranes, etc.), are adapted to drive the food-particles into the cytostome.

After the food has found its way into the cytoplasm, it is digested. In the case of saprozoic forms the dissolved food substances are assimilated directly, but in phagotrophic protozoa the food-particles become enclosed in a drop of fluid, to form a so-called FOOD-VACUOLE, within which digestion takes place. The food-vacuoles circulate in the cytoplasm until digestion is completed, after which the undigested residue is extruded from the body. In amœboid organisms they are ejected at any point of the body but in ciliates they are discharged through a special anal pore, the CYTOPYGE, which is usually situated in the posterior end of the body (Fig. 16, a).

Many protozoa, especially freshwater forms, are provided with special structures, known as CONTRACTILE VACUOLES (Fig. 18, b, c, f-h), which are excretory in function and serve to eliminate from the body excess of water and various soluble products of metabolism. Contractile vacuoles are generally formed in the endoplasm by small droplets of fluid fusing into a single spherical drop, which discharges its contents through the wall of the body. As a rule the vacuole pulsates continuously, being alternately filled (diastole) and emptied (systole) at regular intervals. With the exception of the ciliates, contractile vacuoles do not occur in parasitic protozoa.

Little is known regarding the metabolic processes of protozoa but those organisms which have been more thoroughly studied appear to possess enzyme systems, which are closely related to those of higher animals. Carbohydrates, fats and proteins, ingested as food particles, are broken down by means of appropriate enzymes and the products of decomposition are assimilated. In addition to such basic food substances, many protozoa require for their maintenance small amounts of accessory food factors, some of which are identical with the vitamins essential to mammalian nutrition. Other substances, though not indispensable, serve to stimulate and accelerate growth.

A comparative study of the physiology of the protozoa shows that there is every gradation from forms possessing chlorophyll and capable of photosynthesis, through forms with a saprozoic mode of nutrition, to forms feeding phagotrophically on formed particles. While in the more primitive organisms these are taken in by means of pseudopodia, in the more highly developed organisms they are

ingested through a cytostome. The available evidence indicates that in the course of their evolution the protozoa tend to lose the power of synthesis from inorganic compounds and to become dependent on pre-formed organic matter for their nutrition.

The substances produced by metabolism are not wholly used up for the growth and maintenance of the organism but some of them may be stored for future use. Such reserve food matter occurs in the cytoplasm of protozoa in the form of granular or crystalline inclusions and may be represented by carbohydrates (e.g. glycogen in the cysts of parasitic amœbæ: Pl. I; Fig. 4, e), fats (e.g. cholesterol in coccidia) and proteins (e.g. chromatoid bodies in the cysts of amœbæ: Pl. I; Fig. 4, e). Many protozoa (e.g. trypanosomes) contain in their cytoplasm reserve nuclear material in the form of so-called *volutin* granules, representing ribonucleic acid. Other products of metabolism stored in the cytoplasm play no further rôle in the life of the protozoa (e.g. pigment granules in malaria parasites: Pl. III).

**Respiration.**—The energy required for the vital activities of organisms is provided in the long run by oxidation of foodstuffs. Protozoa are dependent upon the surrounding medium for their supply of oxygen, different species varying considerably in their requirements in this respect. While some, known as *aerobes*, live in a medium rich in free oxygen, others (*anaerobes*) cannot live under such conditions but are adapted to a habitat in which the oxygen tension of the surrounding medium is very low. Aerobic protozoa are represented by forms living in water or—in the case of parasites—in the blood. In such organisms the oxygen, which is dissolved in the surrounding medium, diffuses into the body, where it takes part in the metabolic reactions, which end in the release of energy from the breakdown of foodstuffs. In the course of this process there is liberation of carbon dioxide, which diffuses out into the surrounding medium. Anaerobic forms occur among protozoa inhabiting the slime at the bottom of stagnant pools (so-called *sapropelic* protozoa), in sewage disposal plants, and in those leading a parasitic existence in the lumen of the intestine of higher animals. Such protozoa, which do not flourish in the presence of oxygen, obtain energy by other means, such as molecular rearrangements (e.g. from glucose to lactic acid) or oxido-reductions (e.g. from glucose to carbon dioxide and alcohol).



## CHAPTER 2

# CLASSIFICATION OF THE PROTOZOA

### GENERAL PRINCIPLES

If living organisms are examined and compared, it will be found that they can be arranged in groups, each comprising forms having similar structural features or characters but differing morphologically from other groups. These groups do not as a rule form a continuous chain but are separated from each other by larger or smaller gaps.

The branch of biology devoted to the arrangement or *classification* of organisms into such groups is known as *systematics*, the groups themselves representing *systematic units*, the principles governing the definition of which form the object of *taxonomy*, while the terminology employed in systematics is known as *nomenclature*.

The fundamental systematic unit is the **SPECIES**, which represents a group consisting of individuals with a few common constant characters that clearly differentiate them from individuals in other similar groups. Though there is no universal criterion for the definition of a species, it is usual to separate allied groups of animals as species when the structural characters distinguishing them do not intergrade, i.e. when there is a morphological gap between the groups and intermediate or transitional forms are absent.

Starting from species as basic divisions, systematic units of higher and lower ranks are built into a hierarchy of groups, in an endeavour to produce a system of natural classification which illustrates the *phylogeny*, or mutual affinities and origin of the organisms in question in the course of evolution. In addition to this theoretical aspect, the classification of animals has a practical aim, which is to provide an accurate method for identifying and differentiating individuals, as well as for naming them in accordance with recognized rules of taxonomic procedure.

Related species are grouped together into a higher systematic unit, the **GENUS**. Allied genera constitute a **FAMILY**, and families with features in common are united into an **ORDER**. Orders showing similarities are placed into a **CLASS**, and, finally, classes showing a general resemblance form a **PHYLUM**. In addition to these main

systematic groups, other subdivisions are sometimes employed, usually by prefixing the names with "sub" for a lower division (e.g. subgenus, subfamily, suborder, etc.) or with "super" for a higher one (e.g. superfamily). Species themselves can also be subdivided into minor or intraspecific units. One of these subdivisions, the **SUBSPECIES**, is used for groups which, though differing from each other in constant morphological features (like species), intergrade, i.e. are connected by transitional forms. When the difference between the units is still slighter they are sometimes designated **VARIETIES**. The system of classification accepted at present both by zoologists and by botanists is based on comparative morphology. However, there exist among animals and plants groups which differ from each other in physiological features only but appear to be identical in structure. The position of such groups, known as **BIOLOGICAL RACES**, will be discussed in Chapter 3.

The scientific names employed for the designation of systematic units in zoology are governed by a definite procedure, in accordance with the *International Rules of Zoological Nomenclature*, which have been drawn up by the International Commission on Zoological Nomenclature, a permanent body which deals with all questions concerning the taxonomy of animals and publishes its resolutions periodically in "Opinions and Declarations of the International Commission on Zoological Nomenclature," issued in London.

The following are extracts of the most essential Rules of Zoological Nomenclature \* together with comments.

Article 1.—"Zoological nomenclature is independent of botanical nomenclature in the sense that the name of an animal is not rejected simply because it is identical with the name of a plant."

COMMENT. Though the generic name of an animal may be the same as that of a plant, it is advisable to avoid such duplication.

Article 2.—"The scientific designation of animals is uninominal for subgenera and all higher groups, binominal for species, and trinominal for subspecies."

COMMENT. Each species is named by a combination of two names, the generic and specific, written in *italics*. This designation is known as binominal, e.g. the human dysentery amœba, belonging

to the genus *Entomæba* and to the species *histolytica*, is designated *Entomæba histolytica*. Subspecies bear three names (trinominal), e.g. human lice belong to two subspecies of the species *Pediculus humanus*, which are designated *Pediculus humanus capitis* (head louse) and *Pediculus humanus corporis* (body louse). When part of a species is separated as a subspecies, the original part also becomes a subspecies, the former receiving a new subspecific name, while the subspecific name of the latter is formed by repetition of its specific name. For example, if the small race of *Entomæba histolytica* is recognized as a subspecies, it will bear the name *Entomæba histolytica hartmanni*, the large race becoming *Entomæba histolytica histolytica* (see Chapter 4). Units above the species (subgenera, genera, families, etc.) bear one name with a capital initial letter, e.g. genus *Entomæba*. When a specific name is repeatedly mentioned in the same publication, it is customary to write only the initial letter of the genus, e.g. *E. histolytica*.

Article 3.—“The scientific names of animals must be words which are either Latin or Latinized.”

Article 4.—“The name of a family is formed by adding the ending *idæ*, the name of a subfamily by adding *inæ*, to the stem of the name of its type genus.”

COMMENT. Example: the family name Trypanosomidæ is derived from the genus *Trypanosoma*.

Article 8.—“A generic name must consist of a single word . . . written with a capital initial letter, and employed as a substantive in the nominative singular.”

COMMENT. Examples: genera *Entomæba*, *Trypanosoma*, *Plasmodium*.

Article 10.—“When it is desired to cite the name of a subgenus, this name is to be placed in parenthesis between the generic and the specific names.”

COMMENT. Example: the mosquito-vector of yellow fever belongs to subgenus *Stegomyia* of genus *Aedes* and is designated *Aedes (Stegomyia) aegypti*. When a generic name has been changed the old name may likewise be placed in brackets, provided it is preceded by an equal mark, e.g. *Eimeria* (= *Coccidium*) *stiedæ*, otherwise the name in parenthesis will be mistaken for a subgenus.

Article 13.—“While specific substantive names derived from names of persons may be written with a capital letter, all other specific names are to be written with a small initial letter.”

COMMENT. However, it is customary in protozoological practice to write *all* specific names with a small initial letter, e.g. *Trypanosoma brucei*.

Article 21.—“The author of a scientific name is that person who first publishes the name in connection with an indication, a definition, or a description.”

Article 22.—“If it is desired to cite the author's name, this should follow the scientific name without interposition of any mark of punctuation; if other citations are desirable (date, *sp.n.*, *emend.*, *sensu stricto*, etc.) these follow the author's name, but are separated from it by a comma or by parenthesis.”

COMMENT. Example: *Tricercomonas intestinalis* Wenyon & O'Connor, 1917 indicates that the species in question was created by the said authors in 1917. When a new species (or other systematic unit) is first proposed, it is designated as such in the original publication, e.g. *Eimeria urnula* sp.n.

Article 23.—“When a species is transferred to another than the original genus or the specific name is combined with any other generic name than that with which it was originally published, the name of the author of the specific name is retained in the notation but placed in parenthesis. . . . If it is desired to cite the author of the new combination, his name follows the parenthesis.”

COMMENT. Example: *Amaba coli* Grassi, 1879 was transferred to the genus *Entamaba* by Casagrandi and Barbagallo in 1895; this change is indicated in the notation *Entomaba coli* (Grassi, 1879) Casagrandi & Barbagallo, 1895.

Article 25.—“The valid name of a genus or species can only be that name under which it was first designated.”

COMMENT. This is the so-called “Law of Priority” and is subject to certain conditions: (a) that the name was published and accompanied by an indication, definition, or description; (b) that the author has applied binominal nomenclature.

Article 32.—“A generic or a specific name, once published, cannot be rejected, even by its author, because of inappropriateness.”

COMMENT. The name *Trypanosoma simia*, given to a parasite naturally infecting pigs, cannot be changed to “*T. suis*” on the grounds that the former is inappropriate.

Article 34.—“A generic name is to be rejected as a homonym when it has previously been used for some other genus of animals.”

**Article 35.**—"A specific name is to be rejected as a homonym when it has previously been used for some other species or subspecies of the same genus."

**Article 36.**—"Rejected homonyms can never be used again. Rejected synonyms can be used again in case of restoration of erroneously suppressed groups."

**COMMENT (Articles 34-36).** A homonym is one and the same name for two or more different things. Synonyms are different names for one and the same thing.

### APPLICATION

The application of the Rules of Zoological Nomenclature can be illustrated by the history of the nomenclature of the flagellate causing Oriental Sore in man. Firth in 1891 called it "*sporozoa furunculosa*," but this is regarded as merely a descriptive medical term; moreover since the first name begins with a small letter and both are in the plural, Firth's name infringes Article 8 and is therefore not valid. In 1903 Wright named this parasite *Helcosoma tropicum*, while in 1904 Marzinowsky and Bogrow independently gave it the name *Ovoplasma orientale*. However, it was soon recognized that the parasite of Oriental Sore belonged to the genus *Leishmania* created by Ross in 1903 for the related parasite of Kala-Azar. Blanchard, in 1904, accordingly transferred the former to this genus under the name *Leishmania furunculosa*. However, Lühe in 1906 pointed out that the specific name "*furunculosa*" was invalid and rejected it, substituting for it the name *tropica*, on grounds of priority. The present systematic position of this flagellate is therefore as follows.

Valid name: *Leishmania tropica* (Wright, 1903) Lühe, 1906.

Chief synonyms: "*sporozoa furunculosa*" Firth, 1891.

*Helcosoma tropicum* Wright, 1903.

*Ovoplasma orientale* Marzinowsky & Bogrow, 1904.

*Leishmania furunculosa* (Firth, 1891) Blanchard, 1904.

### SYSTEMATIC ARRANGEMENT

In the classification of the Protozoa an endeavour is made

to arrange them, as far as possible, in a natural system, illustrating the phylogenetic relationships of the various groups, but since in most cases the affinities of the protozoa can only be determined by a comparison of existing forms, this ideal is not always attainable. At one time it was generally believed that the most primitive forms of protozoa were represented by the amœbæ. However, at present there is a tendency to regard the flagellates as being nearer to the ancestral forms of the protozoa, since they comprise holophytic forms, which, like plants, must have come into existence before animals with a holozoic mode of nutrition. In accordance with this view, the flagellates now occupy the first place in the classification of the protozoa, and are followed by the amœbæ and allied organisms, with which they are connected by transitional forms. Among the flagellates the holophytic forms precede the holozoic ones. As regards the ciliates, they represent the most highly organized group of protozoa. In view of the close similarity of cilia and flagella, it is conceivable that the Ciliophora might have originated from some group of multiflagellate Mastigophora. The exact affinities of the Sporozoa have not been determined but certain subdivisions of this group (e.g. the Coccidiida and the Hæmosporidia) provide evidence of common origin.

The Protozoa are divided into four main groups or classes, the MASTIGOPHORA, the RHIZOPODA, the SPOROZOA and the CILIOPHORA, which differ from each other primarily in the external organs of locomotion. The main divisions of a class are orders, which are subdivided into families, and these into genera. In some cases orders are united into subclasses and/or divided into suborders. Since this book is devoted mainly to protozoa of medical interest, in the classification which follows the groups comprising human parasites are given more prominence than groups containing other types of protozoa, many of which are omitted altogether from the list. Among the genera named those which contain species of protozoa living in man are marked with an asterisk.

### SUBKINGDOM PROTOZOA

#### CLASS I. MASTIGOPHORA.

Protozoa, known as flagellates, whose organs of locomotion are represented by flagella.

## SUBCLASS A. PHYTOMASTIGINA.

Typically holophytic flagellates possessing chromatophores ; mostly free-living forms.

## SUBCLASS B. ZOOMASTIGINA.

Typically holozoic (including saprozoic and phagotrophic) flagellates ; free-living and parasitic forms.

## ORDER 1. Protomonadida.

Flagellates with 1-2 flagella and one nucleus ; mostly parasitic forms.

## FAMILY TRYPANOSOMIDÆ.

Flagellates with 1 flagellum and kinetoplast ; parasitic forms exclusively.

GENERA : \* *Leishmania*, \* *Trypanosoma*.

## FAMILY BODONIDÆ.

Flagellates with 2 flagella, one trailing.

GENERA : *Bodo*, \* *Embadomonas*, *Cercomonas*.

## ORDER 2. Polymastigida.

Flagellates with 4 or more flagella and one or more nuclei.

## SUBORDER Monomonadina.

Flagellates with single nucleus.

## FAMILY TETRAMITIDÆ.

With 4 flagella, one attached, axostyle absent.

GENUS : \* *Enteromonas*.

## FAMILY CHILOMASTIGIDÆ.

With 4 flagella, one trailing, axostyle absent.

GENUS : \* *Chilomastix*.

## FAMILY TRICHOMONADIDÆ.

With 4-6 flagella, one attached to undulating membrane, axostyle present.

GENUS : \* *Trichomonas*.

## SUBORDER Diplomonadina.

Flagellates with 2 nuclei and bilaterally symmetrical body.

GENUS : \* *Giardia*.

## SUBORDER Polymonadina.

Flagellates with many nuclei and flagella ; intestinal parasites of termites.

## ORDER 3. Hypermastigida.

Flagellates with many flagella and single nucleus ; intestinal parasites of termites and cockroaches.

## FLAGELLATE OF UNCERTAIN POSITION.

Amœboid form with features suggesting affinities with flagellates.

GENUS : \* *Dientamœba*.

## CLASS II. RHIZOPODA.

Protozoa whose organs of locomotion are represented by pseudopodia ; body naked or covered with shell.

## ORDER 1. Amœbida.

Protozoa commonly known as amœbæ, with naked body, producing blunt pseudopodia (in some forms also flagella) ; free-living and parasitic forms.

## FAMILY AMŒBIDÆ.

Amœbæ without flagellate phase.

GENERA : *Hartmannella*, \* *Entamœba*, \* *Endolimax*, \* *Iodamœba*.

## FAMILY DIMASTIGAMŒBIDÆ.

Amœbæ with flagellate phase.

GENUS : *Dimastigamœba*.

## ORDER 2. Testacea.

Amœbæ enclosed in simple shell ; chiefly freshwater forms.

## ORDER 3. Foraminifera.

Amœbæ enclosed in simple or chambered shell ; chiefly marine forms.

## ORDER 4. Heliozoa.

Spherical organisms ("sun animalcules") with stiff radiating pseudopodia ; chiefly freshwater forms.

## ORDER 5. Radiolaria.

Organisms with membranous capsule in centre of the body, usually with skeleton ; radiating pseudopodia ; chiefly marine forms.



## CLASS III. SPOROZOA.

Parasitic protozoa, producing by multiple fission young forms (sporozoites) typically enclosed in a cyst (spore); organs of locomotion absent.

## SUBCLASS A. TELOSPORIDIA.

Spore without polar filament.

## ORDER 1. Gregarinida.

Trophozoite extracellular, reproduction by sporogony, giving rise to spore containing sporozoites; parasites of invertebrates (commonly known as gregarines).

## ORDER 2. Coccidiida.

Trophozoite intracellular, reproduction by alternation of schizogony and sporogony, typically giving rise to primary cyst (oocyst) enclosing spores containing sporozoites; parasites of invertebrates and vertebrates.

COCCIDIA: chiefly intestinal parasites.

GENERA: \* *Isospora*, *Eimeria*.

HÆMOGREGARINES: chiefly parasites of circulatory system in vertebrates.

GENERA: *Hamogregarina*, *Karyolysus*, *Hepatozoon*.

## ORDER 3. Hemosporidia.

Intracellular forms, reproduction by alternation of schizogony and sporogony, taking place in vertebrate and invertebrate hosts respectively (alternation of hosts), with production of non-resistant oocyst containing sporozoites; parasites of erythrocytes and cells of reticulo-endothelial system in vertebrates.

GENERA: *Hamoproteus*, *Leucocytozoon*, \* *Plasmodium*.

## SUBCLASS B. CNIDOSPORIDIA.

Spore with polar filament.

## ORDER 1. Myxosporidia.

Parasitic in fishes.

## ORDER 2. Microsporidia.

Parasitic in arthropods and fishes.

## SPOROZOA OF DOUBTFUL POSITION.

## 1. PIROPLASMS.

Parasites living in erythrocytes of mammals, transmitted by ticks.

GENERA : *Babesia*, *Theileria*.

## 2. SARCOSPORIDIA.

GENUS : \* *Sarcocystis*.

## 3. TOXOPLASMS.

GENUS : \* *Toxoplasma*.

## CLASS IV. CILIOPHORA.

Protozoa whose organs of locomotion are represented by cilia.

## SUBCLASS A. OPALINATA.

Mouth absent, two or more similar nuclei ; parasitic in amphibians.

## SUBCLASS B. CILIATA.

Mouth usually present, nuclei of two kinds (macro- and micro-nucleus) ; free-living and parasitic forms, commonly known as infusoria.

## ORDER 1. Holotrichida.

Typically uniformly covered with similar cilia.

GENUS : *Ichthyophthirius* (skin parasite of fish).

## ORDER 2. Heterotrichida.

Cilia near mouth (adoral zone) longer than those on rest of the body.

GENUS : \* *Balantidium*.

## ORDER 3. Entodiniomorpha.

Cilia reduced to several bundles ; chiefly intestinal parasites of ungulates.

## ORDER 4. Peritrichida.

Cilia typically restricted to adoral zone ; free-living and ectoparasitic forms, usually attached.

GENUS : *Trichodina* (parasitic in fish).

## SUBCLASS C. SUCTORIA.

Cilia present only in young stage, adult with tentacles.

GENUS : *Allantosoma* (parasitic in intestine of horse).

## CHAPTER 3

### ECOLOGY OF THE PROTOZOA

THE branch of biology which deals with the relations of living organisms to their environment and to other organisms, is known as ecology. The diversity of physico-chemical conditions in the environment determines the distribution of organisms, which are adapted both morphologically and physiologically to a life in certain places of abode, or HABITATS, where they find the optimum conditions of existence. From the ecological point of view protozoa, in common with other animals, can be divided into two major groups : (1) those leading an independent existence, or free-living protozoa ; and (2) those whose existence is more or less intimately associated with other organisms, known as HOSTS, which furnish the protozoa with food and/or shelter. These two groups differ essentially in their relation to the external environment, represented by the outside world. The free-living protozoa enter into direct relations with the outside world, which represents their immediate and only environment, and are subjected to the influence of external conditions exclusively. Protozoa belonging to the second group are affected in varying degrees both by the external conditions and by those provided by the organisms with which they are associated. They range from forms which depend almost entirely on external conditions to forms commonly known as parasites, which are subjected to the exclusive influence of their internal environment in the host.

#### Distribution of the Protozoa

All the protozoa live in a fluid medium, containing varying amounts of moisture. The free-living forms are found all over the world, inhabiting fresh waters, seas, moist earth and sand, the surface of plants, and organic infusions. The freshwater forms, which find habitats suitable to their requirements everywhere and are freely disseminated in the form of cysts, are cosmopolitan in their geographical distribution. The marine protozoal fauna may vary considerably in different parts of the world, according to the salinity and temperature of the water in different seas. As regards parasitic protozoa, their distribution corresponds to that of their hosts.

The members of various systematic groups of protozoa are not uniformly represented in different environments. Thus the class Sporozoa comprises parasitic forms exclusively, while the other classes contain forms living both in fresh and salt waters, as well as forms leading a parasitic existence. Among the Rhizopoda some orders, like the Radiolaria and the Foraminifera, are almost exclusively marine inhabitants, while the Heliozoa and Testacea occur chiefly in fresh water; on the other hand, the parasitic forms are almost entirely restricted to the Amœbida. Likewise among the Mastigophora some groups, like the Phytomastigina, are mainly free-living forms, whereas parasitic forms occur chiefly among the Zoomastigina. As regards the Ciliophora, or ciliates, they are more abundant in fresh waters than in seas, and also contain numerous parasitic forms.

#### Protozoa Leading a Free Existence

The mode of life of the free-living protozoa is so varied that it can only be dealt with very briefly here. Among the marine protozoa some live at the bottom of the sea, while others swim at various depths or float on the surface. The surface or plankton forms, represented chiefly by Radiolaria and Dinoflagellates, play an important rôle in the economy of the sea, for they serve as food for the larger plankton organisms, which in their turn constitute the main diet of many kinds of fishes (e.g. herrings and mackerel) and even of whales. The Foraminifera and Radiolaria, which occur in enormous numbers in the oceans, provide material for the formation of the crust of the earth. After the death of these organisms their shells and skeletons sink in countless numbers to the bottom of the sea and form a fine ooze ("Globigerina ooze" and "Radiolarian ooze") covering the sea-floor. In the course of geological epochs these deposits gave rise to thousands of feet of solid rock, such as limestone and chalk, which subsequently became exposed on the surface of the earth.

The freshwater protozoa can be divided into several groups, according to the environmental conditions under which they live. Those inhabiting clean waters with little organic matter, very few bacteria and abundant free oxygen, are known as *katharobes* and are rarely encountered. Well oxygenated waters, rich in minerals and containing a small amount of organic matter, are well stocked

with holophytic protozoa, which in this habitat are known as *oligosaprobies*. Waters containing bacteria and fair amounts of decomposing organic matter, undergoing oxidation, are densely populated with protozoa known as *mesosaprobies*. Finally, waters with little or no free oxygen but with an abundance of bacteria, decomposing organic matter and products of putrefaction, harbour protozoa known as *polysaprobies*, which lead an anaerobic existence and, together with other microorganisms present in the slime, constitute the so-called "sapropelic fauna." Under certain conditions the activities of the protozoa in a foul water collection lead to its gradual purification, accompanied by a change of the protozoal population from polysaprobies to mesosaprobies and oligosaprobies. A similar succession of protozoal faunas can be observed under laboratory conditions in hay infusions.

Some of the polysaprobies are coprophilic, showing a predilection for dung, manure, and excrement in general. These so-called *coprozoic* protozoa are encountered in temporary pools contaminated with faecal matter and in the stools of various animals deposited on the ground, including those of human beings and domestic animals. Since they may give rise to confusion with parasitic forms, the coprozoic forms are of some medical importance and will be dealt with more fully in Chapter 8. Coprophilic protozoa are also found in sewage, where they take part in the purification processes.

Free-living protozoa, especially coprophilic forms, also occur in the soil, where they live together with bacteria in minute films and pockets of fluid between the solid particles. When desiccation of this medium takes place, the protozoa encyst and may remain dormant until the soil is moistened again, or they may be scattered by the wind and establish themselves elsewhere.

The free-living protozoa are not only influenced directly by the conditions in the external environment but their existence also depends upon other organisms of the community occupying the same habitat, with which the protozoa are linked in food-chains. While the holophytic protozoa are entirely independent of other organisms for their existence, they themselves may serve as food for other animals. On the other hand, the holozoic protozoa are not only preyed upon by other animals but they depend upon other organisms as a source of food. Thus the phagotrophic forms capture other microorganisms, while the saprozoic forms obtain nourishment from

the decomposed remains of animals and plants. However, unlike those protozoa which live on or in other animals or plants, the true free-living protozoa do not enter into a permanent and intimate association with other organisms.

### Protozoa Living in Association with Other Animals

There is no sharp dividing line between true free-living protozoa and protozoa living in association with other organisms. Free-living protozoa, notably sessile flagellates and ciliates, sometimes attach themselves to the external surface of the body of all sorts of animals and plants indiscriminately. In such cases the organism with which they are associated serves merely as a temporary substratum but does not otherwise affect the protozoa, whose existence is to all intents and purposes independent of the organism to which they happen to adhere. The association is more intimate in the case of protozoa which enter into an inseparable and permanent association with another organism, known as the **HOST**. The hosts may be plants or animals but in the account which follows we shall consider only associations between the protozoa and other animals, including man. As a rule, in these permanent associations the two partners are unequal in size, the protozoon (or microorganism) being considerably smaller than the host (or macroorganism). Moreover, the advantages of the relations between the microorganism and the macroorganism are unilateral. The protozoon is always the interested party, since it invariably benefits from the association with its host, being strictly dependent upon the latter for shelter, food, or both, and being incapable of living apart from the host. As regards the host, which is represented by an animal leading an independent existence, it is either indifferent to or suffers from the presence of the protozoon, though exceptionally it may benefit from the association.

There are various types and degrees of such associations, which are designated by different names, according to the nature of the relationship between the two partners. From the point of view of spatial relations, the protozoa may be divided into **ECTOZOIC** (also **epizoic**) and **ENTOZOIC** forms, according to whether they live on the surface of or inside the body of the host. In these associations the host may provide the protozoon only with lodging or with food as well. In the former case, known as "**SHELTER-ASSOCIATION**"

(French *synécie*), the protozoa are either attached externally to the body of the host or inhabit natural cavities of its body which open to the outside. The host provides these ectozoic protozoa with a lodging, but they obtain nourishment from the external environment. Examples of such an association are provided by protozoa attached to various parts of the body of crustaceans or living in the mantle-cavity of molluscs. Whereas in "shelter-associations" the protozoon is independent of the host as a source of food, in other associations it is entirely dependent upon it for its food supply. Such associations are referred to three categories. When the protozoa share the host's food without causing it any harm, the association is known as COMMENSALISM; when the protozoa feed on the host's own tissues or body fluids and the association is detrimental to the host, it is known as PARASITISM; lastly, when the association is to the mutual benefit of both partners, it is known as SYMBIOSIS.

Both commensalism and parasitism occur among protozoa living either outside or inside the host, the former being known as ecto-commensals or ectoparasites, the latter as endocommensals or endoparasites, as the case may be. An example of ectocommensalism is afforded by certain ciliates which are attached to the anal region of tadpoles and feed on the droppings discharged from the body of their host. Endocommensalism is characteristic of many protozoa living in the alimentary tract of other animals, where they feed exclusively on the intestinal contents, consisting of undigested food particles and microorganisms, which, as far as the host is concerned, represent superfluous matter. The commensal protozoa are thus merely scavengers or messmates, whose presence and activities cause no harm to the host. Examples of ectoparasitism are provided by the flagellate *Costia necatrix* and the ciliate *Chilodon cyprini*, both of which are attached to the skin and gills of various fishes, where they feed on the epithelial cells, frequently causing the death of the host. Endoparasitism is widespread among the protozoa, which invade animals of all classes, living in different parts of the alimentary tract, in the blood stream and in various tissues and organs. Some endoparasitic protozoa wander freely in the medium occupied by them, others are attached to the walls of various organs, while others again are found in or between the cells of the host's tissues. Their feeding habits vary considerably, some using up part of the

food intended for the host itself, others feeding at the expense of the fluid components of the host's body, others again ingesting elements of its tissues. Though the damage is not always evident, the presence of parasitic protozoa harms the host, by depriving it of some of its food, by consuming elements of its body, or otherwise injuring it.

As stated above, the difference between commensalism and parasitism is based on the effect produced upon the host by the protozoa associated with it, this effect being determined chiefly by the mode of nutrition of the latter. Actually, however, the distinction between these two types of association is not clearly demarcated, for in most cases parasitic protozoa are capable of leading a commensal existence, while many harmless commensal protozoa may become true parasites. Commensalism and parasitism are, therefore, sometimes reversible. A distinction must also be drawn between ectozoic and entozoic habits. While in the former case the protozoa enter into direct relations both with the external environment and their host, being equally influenced by both, in the latter case the protozoa are only indirectly affected by the outside world but depend exclusively upon the inner environment provided by the host. Since the protozoa harboured by man are represented exclusively by entozoic forms, we shall deal with endocommensalism and endoparasitism among the protozoa more fully in another section.

We now turn to symbiosis, an association which benefits not only the protozoa but their host as well, on account of which it is sometimes called *mutualism*. The best known example of symbiosis is afforded by flagellates (chiefly of the orders Polymastigida and Hypermastigida) living in the intestine of termites. These insects feed almost exclusively on wood and other substances containing cellulose but are unable to digest their food without the aid of the symbiotic flagellates. The latter ingest particles of wood present in the gut of the insect and make the products of decomposition of cellulose available for the nutrition of the host. It has been demonstrated, by artificial elimination of the flagellates from the gut of the termites, that these insects soon die of starvation in the absence of the protozoa.

**Terminology.**—In the account given above, various types and degrees of associations between the protozoa and their hosts are



designated by different names, according to the nature of the relationship between the two partners, the term *parasitism* being used in a restricted sense, to indicate a special type of association. Unfortunately, however, this terminology is not strictly adhered to, for *parasitism* is also generally used as a comprehensive term, to indicate all kinds of associations in which one organism (in our case a protozoon) lives on or in another organism, which serves as its host. In all these cases the protozoa are commonly described as being *parasitic*, while the associations themselves—irrespective of their nature—form the subject-matter of the branch of biology known as Parasitology. In medical protozoology, therefore, the term *parasite* is used with two different meanings: (a) as a general term, loosely applied to all protozoa living in man; and (b) as a restricted term, indicating an association in which the protozoa live at the expense of and are harmful to the host. In the general account of the protozoa we have already used the term “parasitic” in the broad sense of the word.

Among other terms used in parasitology the following should be mentioned. An animal (the host) harbouring protozoa, is said to be *infected* with these organisms, their presence in the host being known as *infection*. Parasitic protozoa causing recognizable changes or disease in the host are called the *causative organisms* of the disease; they are said to be *pathogenic* and are sometimes referred to as *pathogens*, while the degree of their noxiousness or pathogenicity is called *virulence*. The names of diseases caused by parasites are generally formed by the addition of the suffix *-iosis* or *-iasis* to the scientific name of the group to which the causative organism belongs. Thus the terms *amæbiasis*, *coccidiosis* and *piroplasmosis* denote diseases caused by amæbæ, coccidia and piroplasmids respectively. In addition to these general terms, some protozoal diseases have special names, e.g. certain diseases provoked by species of the genera *Plasmodium*, *Leishmania*, *Trypanosoma* and *Theileria* are known as malaria, Kala-Azar, Dourine and East Coast Fever respectively.

#### Economic Importance of the Protozoa

Many of the protozoa are of economic importance in various spheres of human activity. While some free-living protozoa play a positive rôle, by contributing something that can be utilized by

man, the rôle of parasitic protozoa is entirely negative, since the diseases provoked by them cause nothing but economic loss.

Free-living protozoa play an important part in geology, mining engineering, public health, fisheries, and probably agriculture. In some geological formations the rocks are composed almost entirely of the fossil remains of Foraminifera and Radiolaria, thus the limestones of which the Egyptian pyramids were built had been formed by Foraminifera. Furthermore, in the petroleum industry Foraminifera, found in samples obtained in the drilling of wells, provide indications of oil-bearing zones. Coprophilic protozoa are of some public health importance; on the one hand, on account of the part they play in promoting sewage purification in sewage disposal plants; on the other hand, as indicators of the degree of pollution in water supplies. The agricultural importance of free-living protozoa is not quite clear, but it is possible that those living in the soil serve some useful purpose. Finally, marine protozoa present in the plankton are of some importance in fisheries, since they contribute directly or indirectly to the nutrition of fishes.

Parasitic protozoa occur in representatives of all classes of animals, both terrestrial and aquatic, in most parts of the world. Their economic importance is due to the fact that many of them cause serious diseases in animals. Those affecting domesticated animals and useful wild animals, such as food, fur-bearing and game animals, as well as those kept as pets or in menageries, are of practical importance in animal husbandry, fisheries, agriculture, sericulture, apiculture and other branches of economic zoology. While most protozoal diseases of lower animals, especially those of livestock, belong to the domain of veterinary medicine, some of these infections have a direct bearing on human disease, either because the protozoa in question are common to man and the lower animals or because they are closely related to those parasitic in man. In the former case they are of direct interest in human medicine, in the latter they provide material for a comparative study of human and animal diseases. Finally there are the protozoa living in man, either as commensals or as parasites, and causing various important diseases. The purely human protozoa and those common to man and other animals form the subject matter of medical protozoology proper and will be considered in subsequent pages,

while the practical importance of protozoal diseases in various groups of lower animals are illustrated by the following examples.

**Insects.**—Microsporidia of the genus *Nosema* give rise to a devastating disease, known as “pébrine,” in silkworms (*Bombyx mori*), different tissues and organs of which are attacked by the parasite, causing the death of the caterpillars. Another species of *Nosema*, which is parasitic in the stomach of the honey-bee, causes in these insects “*Nosema*” disease, which may be accompanied by diarrhoea and is sometimes fatal.

**Fishes.**—Myxosporidia are common in edible freshwater and marine fishes, in which they invade various organs and tissues and cause disease. Thus, *Myxosoma* attacks the cartilaginous skeletal elements of young trout, being responsible for the “twist disease,” which often causes considerable destruction in hatcheries. *Myxobolus* invades the muscles and various organs of barbel, causing “boil disease,” with the production of tumours, which is fatal to the fish. Another destructive disease is caused by the ciliate *Ichthyophthirius*, which is parasitic in the skin and gills of freshwater fishes (e.g. pike and tench), producing numerous pustules. Among ectoparasitic protozoa may be mentioned the flagellates *Costia* and *Oodinium*, which are attached to the skin and gills respectively of marine fishes, causing considerable mortality among them.

**Birds.**—Among the protozoal diseases of poultry, coccidiosis due to species of *Eimeria* is very common in the domestic fowl, causing considerable losses to breeders in different parts of the world. The disease, which is manifested by diarrhoea, is usually fatal to chickens. Turkeys are frequently affected by “Blackhead,” a form of enterohepatitis caused by an intestinal flagellate, *Histomonas meleagridis*, which invades the liver and may be fatal to these birds. In North America ducks suffer from malaria caused by *Leucocytozoon*. Pigeons are subject to a severe form of coccidiosis, brought about by an *Eimeria*, and to infection with an intestinal flagellate of the genus *Trichomonas*, which may penetrate into various organs, with fatal results.

**Mammals.**—Protozoal diseases occur in most domestic mammals, especially in warm and tropical countries. Cattle are liable to an intestinal disorder, known as “red dysentery,” which is a form of coccidiosis caused by *Eimeria zürnii*, with a high mortality among calves. Bovines also suffer from a venereal disease caused by

*Trichomonas fetus*, which invades the genital tract and is responsible for sterility and abortion in cows. However, the most important bovine diseases are due to trypanosomes and piroplasms, blood-inhabiting protozoa belonging to the classes Mastigophora and Sporozoa respectively. In tropical Africa outbreaks of trypanosomiasis are responsible for serious losses among cattle, while a piroplasm of the genus *Theileria* is the cause of East Coast fever, an acute disease with a high mortality rate, characterized by hæmoglobinuria. Other forms of piroplasmosis in cattle are caused by species of the genus *Babesia*, one of which, *B. bigemina*, is responsible for "Redwater" or Texas fever, occurring in warm countries, while another species, *B. bovis*, produces hæmoglobinuric fever in European cattle. Sheep and goats are also subject to trypanosomiasis and piroplasmosis. In Africa pigs frequently succumb to a virulent form of trypanosomiasis due to *Trypanosoma simia*. The pig also harbours in its intestine the ciliate *Balantidium coli*, which is transmissible to man. A form of trypanosomiasis, known as Surra, is widespread among camels and horses in warm countries, especially in Asia and North Africa. Horses are also subject to a venereal form of trypanosomiasis, known as Dourine, which is caused by *Trypanosoma equiperdum* and affects the genital organs. The most important protozoal disease of dogs is malignant jaundice, a form of piroplasmosis caused by *Babesia canis*, occurring in different parts of the world. Domestic and wild rabbits are frequently infected with a coccidium, *Emeria stiedæ*, which invades the liver, causing a severe and sometimes fatal disease.

### THE ENTOZOIC PROTOZOA OF MAN

General.—The protozoa associated with man are represented exclusively by entozoic forms. Since the entozoic habit has a number of peculiarities which distinguish it from other modes of existence, this phenomenon will be considered more closely. As already briefly mentioned, the main difference between free-living and ectozoic organisms, on the one hand, and entozoic organisms, on the other, lies in their relation to the external environment represented by the outside world. While organisms of the first two groups are entirely or partly dependent on external conditions,

those belonging to the third group have become completely emancipated from the direct influence of the outside world, their relations with which are regulated indirectly, through the medium of the host. The body of the host therefore constitutes the immediate habitat of the entozoic organism and comprises all the factors, including nutrition, which determine its existence. The entozoon benefits from the association by being relieved of the necessity to adjust its functions to fluctuations in the external environment, which is replaced by a more or less constant internal environment provided by the host. However, life within the body of another animal involves relations which are not evident in the ecology of free-living organisms. While in the latter case the effect of the external environment is paramount, in the former case the entozoon and its host enter into direct physiological relations with each other, the vital activities of one partner affecting the functions of the other. In fact there is a mutual antagonism between the entozoon and its host, the effects of which upon either of the partners depend upon the degree of adaptation established between them. In the case of commensalism, an equilibrium is established, the entozoon living in harmony with the host, without causing it any injury. In the case of parasitism, the amount of harm caused by the entozoon depends upon the effectiveness of the host's resistance to the activities of the parasite. The interaction between the host and the entozoon, known as **HOST-PARASITE RELATIONSHIP**, is of fundamental importance in parasitology. A knowledge of these relations is indispensable for the understanding of the behaviour of the entozoic protozoa and of the reactions of the human host to the infection, both of which contribute to the pathogenesis of the protozoal diseases of man.

**Species.**—The exact number of species of protozoa living in man is variously estimated, according to the interpretation attached to their validity by different authorities. In this book we accept about twenty-seven species, with certain reservations mentioned in the appropriate places. A list of the entozoic human protozoa, arranged in systematic order, is given below. Among these species a dozen (Nos. 1, 14–18, 21–25, 27) are pathogenic, causing recognizable diseases; ten (Nos. 2–11) are harmless commensals; while opinions regarding the pathogenicity of four species (Nos. 12, 13, 19, 26) are divided.

The Entozoic Protozoa of Man

RHIZOPOOA

- |                                   |  |
|-----------------------------------|--|
| 1. <i>Entamoeba histolytica</i> . | 14. <i>Leishmania donovani</i> .                           |
| 2. <i>E. coli</i> .               | 15. <i>L. tropica</i> (including <i>L. brasiliensis</i> ). |
| 3. <i>E. gingivalis</i> .         | 16. <i>Trypanosoma gambiense</i> .                         |
| 4. <i>Endolimax nana</i> .        | 17. <i>T. rhodesiense</i> .                                |
| 5. <i>Iodamoeba bütschlii</i> .   | 18. <i>T. cruzi</i> .                                      |
- (*Dientamoeba*: see under MASTIGOPHORA).

SPOROZOA

19. *Isospora belli*.  
 20. *I. hominis* (?)  
 21. *Plasmodium falciparum*.  
 22. *P. vivax*.  
 23. *P. malariae*.  
 24. *P. ovale*.  
 25. *Toxoplasma hominis*.  
 26. *Sarcocystis lindemanni*.

MASTIGOPHORA

6. *Dientamoeba fragilis*.  
 7. *Enbodomonas intestinalis*.  
 8. *Chilomastix mesnili*.  
 9. *Enteromonas hominis*.  
 10. *Trichomonas hominis*.  
 11. *T. tenax*.  
 12. *T. vaginalis*.  
 13. *Giardia intestinalis*.

CILIOPHORA

27. *Balantidium coli*.

HOST-PARASITE RELATIONSHIP

The association between the entozoic protozoa and man can be divided into the following four phases: (i) penetration of the parasite into the human organism; (ii) its development in the human body; (iii) its emergence from the human body; and (iv) its sojourn outside the human body, which may take place either (a) in the external environment or (b) in a second host. The life-cycle of the protozoa comprises a series of stages of development, adapted to the varying conditions of existence in the successive habitats through which they have to pass. We shall first consider the development of the protozoa in the human host, then their behaviour after emerging from the latter, and finally turn to the method of infection of new human hosts.

Life-Cycles

The activities of the entozoic protozoa are primarily concerned with nutrition and reproduction, which ensure their individual existence within the given human host, and secondarily with

providing for the dispersal of their descendants to new individuals of the host, thereby ensuring their survival as species. These two processes, known as the *multiplicative* and *propagative* phases of the life-cycle, succeed each other in the order indicated. In the course of their development within the human body the entozoic protozoa lead a sheltered existence under optimum conditions, but when the propagative stages leave the human host they are exposed to adverse conditions which necessitate special adaptations. The interval between the time when the protozoa leave the body of the old host and the time when they are transferred to a new one is passed either in the external medium or in a secondary (invertebrate) host. The different conditions in these two habitats have evoked corresponding adaptations on the part of the protozoa. The problem is solved by the production, in the former case, of encysted forms capable of resisting the external conditions; in the latter case, of forms which are able to continue their development in the secondary host, from which they are transferred to a new human host.

The life-cycle of the human entozoic protozoa varies with different species, in accordance with the method of transfer to new hosts.

In the simplest case, represented by most of the intestinal protozoa, the life-cycle is straightforward, without an alternation of generations, the protozoa simply multiplying within the same host (with or without syngamy), and from time to time forming cysts, which are discharged from the host with the *faeces*. A more complicated development occurs in the coccidia, represented in man by *Isospora*; in the life-cycle of these parasites there is an alternation of generations without an alternation of hosts: a period of asexual multiplication by repeated schizogony is succeeded by a period of sexual multiplication, or sporogony, all taking place in the same host and terminating in the formation of encysted forms, known as oocysts, which escape from the body of the host with its *faeces*. In the life-histories considered above, which are characteristic of the intestinal protozoa, the infective stage is represented by resistant forms (cysts) which remain outside the body of the host until taken up by another human being, in whose alimentary tract they excyst or hatch, liberating active forms, which initiate an infection in the new host.

In entozoic protozoa living in the blood and in the reticulo-endothelial system, exposure to the external environment and the consequent necessity of forming resistant stages (cysts) are eliminated, owing to the intervention of a secondary host, or vector, represented by a blood-sucking insect. In the blood-inhabiting flagellates, or hæmoflagellates, comprising the trypanosomes and leishmanias, the life-cycle involves an alternation of hosts without an alternation of generations, the entire development consisting of asexual multiplication with a succession of stages, some of which occur in the vertebrate host, others in the insect-host. In the blood-inhabiting Sporozoa represented by the malaria parasites, the life-cycle is characterized by an alternation of generations combined with an alternation of hosts. Thus in the human host the parasites multiply asexually by repeated schizogony and produce sexual forms (gametocytes), which are the only forms capable of developing in the insect-host; when the blood of a malarial patient is ingested by a mosquito, the gametocytes taken up with the meal go through the sexual phase of their life-cycle in the body of the latter. In the case of both the hæmoflagellates and the malaria parasites, the infective stages, represented by active forms, are produced at the end of the life-cycle in the insect-host, which transfers them to a new human host.

**Definition of hosts.**—Here it should be mentioned that there is no general agreement regarding the terms used to denote the hosts of those parasites in whose life-cycle there is an alternation of hosts. Some authorities refer to the host in which the parasite undergoes sexual development and reaches maturity, as the *final* or *definitive* host, while the host in which its asexual development takes place is said to be the *intermediate* host. Others distinguish the host according to the phylogenetic relations of the parasite, using the term *final* host for the type of animal, in which the ancestors of the parasite in question underwent their entire life-cycle, and reserving the name *intermediate* host for the vector, which was subsequently introduced into the life-cycle as a secondary host. However, in the case of protozoa, neither of these criteria affords a satisfactory solution of the question. Thus, there is no evidence of a sexual cycle among the hæmoflagellates (trypanosomes and leishmanias), while in the malaria parasites sexual development takes place both in the vertebrate host (gametocyte production) and in the mosquito (sporogony). Furthermore, from the available data there can be



little doubt that in the course of evolution the hæmoflagellates have evolved from flagellates restricted to insects, whereas the malaria parasites have originated from coccidia of vertebrate animals. If the criterion of sexual development is applied to these organisms, in the case of the hæmoflagellates there would be no indication which of the hosts, man or insect, is the final host and which is the intermediate one, while in the case of the malaria parasites there would be some justification for accepting the insect-vector as the final host and man as the intermediate host. However, if the phylogenetic criterion is applied, we should have to regard the insect-vector as the final host and man as the intermediate host of the hæmoflagellates, and to reverse the rôles in the case of the malaria parasites.

To avoid this ambiguity we propose to adopt non-committal definitions for all cases of human protozoal infections and to designate the vector as the intermediate host, reserving the name final host for man and other mammals.

### Transmission

The human entozoic protozoa are transmitted from the original host to other human beings in various ways, which differ according to whether the life-cycle of the protozoa in question is MONOGENETIC, i.e. takes place in one host, or DIGENETIC, i.e. divided between two hosts, the final one, represented by man, and an intermediate host, represented by a blood-sucking insect. In the former case the protozoa are transmitted from man to man directly, in the latter indirectly, with the intervention of the intermediate host, or vector. There are several methods of direct and indirect transmission. The protozoa living in the mouth (*Entamæba gingivalis*, *Trichomonas tenax*) and in the genital tract (*Trichomonas vaginalis*), which have no special propagative stages and do not necessarily escape from the human body, are transferred from man to man by contact between two individuals, in the former case probably when kissing, in the latter case during the sexual act. In the case of the intestinal protozoa (amœbæ, flagellates, coccidia and ciliates), the propagative stages, represented by cysts, escape from the body of the original host and are taken up by a new human host by ingestion of food or drink contaminated by the cysts. When there is an alternation of hosts, the propagative stages of the protozoa developing in man are taken up by the insect in the act of feeding, after which they

undergo a cycle of development in the body of the intermediate host, terminating in the production of infective stages of the parasite. These are transmitted to a new human host by various methods, according to their localization in the vector. Thus, in the case of *Trypanosoma cruzi* the infective forms, which are produced in the hindgut of the insect host, are deposited with its infected feces on a mucous membrane (e.g. the conjunctiva), whence they penetrate into the body of the human host. This is known as the CONTAMINATIVE method of transmission. In other blood protozoa the infective forms develop in the anterior region of the alimentary tract (species of *Leishmania*) or in the salivary glands (*Trypanosoma gambiense*, *T. rhodesiense* and species of *Plasmodium*) of the insect-vector, which introduces them into the skin of the human host in the act of biting. This method of transmission is known as INOCULATIVE.

In all the digenetic protozoa infection of a new human host can only take place after the parasite has completed its development in the intermediate host and produced the infective forms. This mode of transmission is termed CYCLICAL, to distinguish it from the so-called MECHANICAL mode of transmission, which may be effected by the transfer of blood protozoa from one man to another through the agency of various blood-sucking insects, in the mouth-parts of which the parasites merely survive without undergoing any further development.

Among the direct methods of transmission should be mentioned HEREDITARY or CONGENITAL transmission from mother to fœtus, which usually takes place through the placenta. It is commonly observed in infections with *Toxoplasma* and has also been reported in malaria and Sleeping Sickness.

From the foregoing account it is seen that the main sites through which the entozoic protozoa gain access to a new human host, or the PORTALS OF ENTRY, are (a) the mouth (*per os*), in the case of intestinal organisms; (b) the integuments, in the case of blood-inhabiting organisms; and (c) the placenta, in the case of toxoplasms.

The chances of an entozoic protozoon finding a new human host are in general precarious, the success being ensured in varying degrees by the different methods of transmission. In the case of the intestinal protozoa the chances are fairly remote, for among the enormous numbers of cysts discharged with the feces only

relatively few succeed in contaminating food and in being accidentally ingested by human beings, whereas the majority of cysts perish without finding a new host. The chances of successful infection are much better in the case of *Trypanosoma cruzi*, since the insect-vector may deposit its infected faeces directly upon the vulnerable parts of the human body. A further improvement in the method of transmission is found in the leishmanias, in the trypanosomes causing Sleeping Sickness and in the malaria parasites, all of which are introduced directly into the human body through the bite of the insect-vector.

### Host-Restriction

In common with free-living organisms, which are restricted in their distribution to certain well-defined habitats, where they find suitable conditions of existence, entozoic protozoa are adapted to life within a limited range of hosts, which provide them with favourable physico-chemical conditions and food, but are unable to establish themselves in other animals. This preference for certain hosts is known as HOST-SPECIFICITY or HOST-RESTRICTION, the latter term being more suitable, in view of the different connotations of the word "specificity" in biology. Among the entozoic protozoa some (*monoxenous* forms) are restricted to a single host, others (*euryxenous* forms) have a wide range of hosts, and others again (*stenoxenous* forms) occupy an intermediate position, being capable of living in a few hosts only. As a rule, the final hosts of a given entozoic organism belong to closely related groups of animals, but the intermediate hosts are zoologically widely separated from the final hosts, on account of which digenetic protozoa may also be called *heteroxenous* forms. Protozoa capable of living in more than one final host frequently show a preference for one or some of them, which are more commonly infected with these organisms. They are known as the PRINCIPAL hosts, while those which are of secondary importance are called SUPPLEMENTARY hosts. Those hosts, in which the protozoa may occur only occasionally under natural conditions or which they are capable of infecting under experimental conditions, are said to be INCIDENTAL hosts.

In general, host-restriction represents a physiological adaptation on the part of the protozoa to conditions in certain hosts, and it may or may not be correlated with morphological distinctions. Thus differences in host-restriction are observed not only between

morphologically recognizable species of protozoa but also among different populations or strains of the same species, which are morphologically indistinguishable. Such strains, which differ only in physiological or biological characters and are known as BIOLOGICAL RACES, will be dealt with more fully below.

The entozoic protozoa of man exhibit various degrees of host-restriction. The malaria parasites (*Plasmodium*) are rigidly restricted to man, though some of them are infective to anthropoid apes which themselves may harbour species of *Plasmodium* indistinguishable from the human parasites, probably representing biological races. Other human protozoa have a wider range of hosts. Thus the dysentery amœba, *Entamoeba histolytica*, is common to man and some monkeys, which are its principal and supplementary hosts respectively, while rats and dogs, which are sometimes naturally infected with this parasite, and cats, which are susceptible to artificial infection, represent incidental hosts. Among the trypanosomes causing Sleeping Sickness, *Trypanosoma gambiense* is restricted to man but goats and pigs are probably incidental hosts; as to *T. rhodesiense*, its principal host is man, while antelopes represent supplementary hosts. On the other hand, the trypanosome causing Chagas' disease, *T. cruzi*, is commonly found in armadillos and some other wild mammals but less frequently in man. In this case, therefore, the armadillo is the principal host, whereas man is a supplementary or incidental host. The position of the intestinal ciliate, *Balantidium coli*, is somewhat similar, for it occurs chiefly in the domestic pig, which should be regarded as its principal host, and only incidentally in man. Among the leishmanias different species and races show a wide variation in their range of hosts. Thus, man is the only known host of *Leishmania donovani* in India, but in the Mediterranean area the dog is a supplementary host of this parasite. As regards *L. tropica*, while in some areas man is the principal host, in others he is only a supplementary host, the principal host being represented by gerbils. The widest range of hosts is found in the case of *Toxoplasma*, which occurs naturally not only in man but in many other mammals, as well as in birds. Among the incidental hosts of trypanosomes and leishmanias should be mentioned various rodents and other mammals which can be infected experimentally with these parasites.

Among the lower animals harbouring human entozoic protozoa

under natural conditions, some represent the source from which man may acquire his infection. Such animals serve as RESERVOIRS of human infections and are known as RESERVOIR HOSTS, while those diseases of lower animals which are communicable to man are called ZONOSSES. Thus, balantidiosis (*Balantidium coli*), certain types of Oriental Sore (*Leishmania tropica*) and of Kala-Azar (*L. donovani*) are common to man and other mammals and therefore represent zoonoses. The reservoir hosts of the first disease are pigs, those of the second are gerbils, and those of the third are dogs. The reservoir hosts of Chagas' disease (*Trypanosoma cruzi*) and of the acute form of Sleeping Sickness (*T. rhodesiense*) are armadillos and antelopes respectively; they differ from the reservoir hosts of zoonoses in that the infection in them produces no symptoms of disease. Various types of host-restriction among the pathogenic human protozoa are shown in Table 1.

TABLE I  
HOST-RESTRICTION AMONG THE PATHOGENIC PROTOZOA  
OF MAN

(Animals marked with an asterisk are not naturally infected.)

HOSTS PROTOZOA	FINAL				INTER- MEDIATE
	PRINCIPAL	SUPPLEMENTARY	INCIDENTAL	RESERVOIR	
<i>Entamoeba histolytica</i>	man	monkeys	rat, dog, cat *	rat (?)	none
<i>Leishmania donovani</i>	man	dog	} mice * hamsters,* etc.	dog	} sandflies
<i>L. tropica</i>	man, gerbils	gerbils, dog, man		gerbils	
<i>Trypanosoma cruzi</i>	armadillos	man, dog	rodents *	armadillos, dog	Reduviid bugs
<i>T. gambiense</i>	man		goat, pig, rodents *	goat, pig(?)	} tsetse- flies
<i>T. rhodesiense</i>	man	antelopes	rodents,* etc.	antelopes	
<i>Plasmodium</i> spp.	man		apes	none	Anopheline mosquitos
<i>Toxoplasma</i>	mammals, birds	man		?	?
<i>Balantidium coli</i>	pig	man, monkeys		pig	none

### Localization

The distribution of entozoic protozoa in the body of the human host varies considerably, the sites of infection being determined by the adaptation of different species and races of protozoa to life in definite organs and tissues of the host (TISSUE-SPECIFICITY). Some protozoa are more or less limited in their distribution to different systems of organs, within which they may be restricted to definite situations. Other protozoa are localized in separate organs or tissues. Thus, many human protozoa live in the alimentary tract, some of them being restricted to the mouth (*Entamoeba gingivalis*, *Trichomonas tenax*), one occurring in the duodenum (*Giardia intestinalis*), and others inhabiting the large bowel (the intestinal amœbæ and flagellates, the ciliate *Balantidium coli*). An important group of protozoa are found mainly in the circulatory system. These include the malaria parasites (species of *Plasmodium*) and trypanosomes (species of *Trypanosoma*). Other protozoa are restricted to elements of the reticulo-endothelial system in different parts of the body (e.g. species of *Leishmania*), to the muscles (Sarcosporidia) and to the genital tract (*Trichomonas vaginalis*). In addition to the principal sites of infection, some protozoa may extend to other parts of the body, either in the normal course of their life-cycle, or as the result of secondary invasions. Thus among the blood-inhabiting forms, *Trypanosoma cruzi* normally multiplies in the muscles, while the malaria parasites undergo certain stages of development in the liver cells; *Entamoeba histolytica* may produce metastatic infections of the liver and other organs, and both this species and *Balantidium coli*, which usually inhabit the lumen of the intestine, may invade its wall. The entozoic protozoa of man also differ in the position they occupy in relation to the histological elements of the host's tissues. Some are *extracellular*, living in natural cavities of the body, like most of the intestinal protozoa, which are found in the lumen of the gut, and the trypanosomes, which are free in the blood stream. Others, like *Entamoeba histolytica* and *Balantidium coli*, may penetrate between the cells of the intestinal wall and occupy an *intercellular* position. Others again lead an *intracellular* existence, being enclosed within various cells of the host's tissues; thus the malaria parasites are found inside the erythrocytes, leishmanias in the macrophages, and *Trypanosoma*

*cruzi* invades the muscle fibres, while the coccidia (*Isospora*) probably inhabit the epithelial cells of the intestine.

The host-restriction and tissue-specificity of the entozoic protozoa of man are fully dealt with in Part II.

### Biological Races

It has already been briefly mentioned that, in addition to recognizable species, the entozoic protozoa comprise so-called BIOLOGICAL RACES, representing subdivisions within a species, which are morphologically indistinguishable but differ from each other in physiological or biological features only. In general, biological races may be said to differ in various aspects of their host-parasite relations. Some races manifest a more or less rigid host-restriction (either to the final or to the intermediate host), others give rise to different diseases in the same host. In the latter category are also races which have a distinct localization in definite organs or tissues, i.e. differing in tissue-specificity, as well as races showing varying degrees of virulence or pathogenicity for the host.

Whereas zoological classification of species is based on morphological differences between animals, in medical and veterinary protozoology the clinical and pathological aspects of protozoal infections have unduly influenced the classification of the entozoic protozoa, with the result that morphologically identical forms, which conform to the concept of biological races, are in most cases described as independent species. The elevation of such forms to the rank of species exaggerates the degree of their systematic distinction and obscures the close affinity between them. Although we have retained most of the existing names for forms which differ in physiological characters, we regard this classification as provisional, until the independent systematic position of biological races is more generally recognized.

Biological races are common among the blood-inhabiting protozoa of man and other mammals. The best-known examples are found among three species of closely related trypanosomes, *Trypanosoma gambiense*, *T. rhodesiense*, and *T. brucei*, which are morphologically indistinguishable but differ in host-restriction, the first two being human parasites, while the third is incapable of infecting man but is restricted to lower mammals. On the other hand, *T. gambiense* and *T. rhodesiense* differ from each other in their

effect upon the human host. Whereas the former produces a chronic type of Sleeping Sickness, the latter provokes an acute type of this disease. It is evident that these trypanosomes, though assigned to three independent species, actually represent biological races of one and the same species. The next group to be considered are the leishmanias, which cause two main types of disease in man: one, a generalized visceral infection known as Kala-Azar, is caused by *Leishmania donovani*; the other, a localized cutaneous infection known as Oriental Sore, is caused by *L. tropica*. Here again the criteria for the classification of these parasites are purely medical, for these two flagellates are morphologically identical, the only clue to the "species" to which they belong being provided by the clinical picture of the case from which they originate. They are therefore undoubtedly biological races of the same species, differing in tissue-specificity. Another example of biological races is provided by the malaria parasites. Anthropoid apes harbour three types of malaria parasites, which are morphologically indistinguishable from the three species commonly found in man, viz. *Plasmodium falciparum*, *P. vivax* and *P. malariae*. However, the human and ape parasites manifest a fairly high degree of host-restriction. Though the anthropoid parasites have been given separate names, it is obvious that the human and simian parasites are limited to three species, each comprising two biological races, one adapted to man, the other to apes.

Biological races of entozoic protozoa are equivalent to "types" occurring among pathogenic bacteria, which also differ in host-restriction, in the type of disease produced in the same host, or in virulence. In the case of bacteria it has been demonstrated that the various pathological manifestations which they produce in the host are correlated with differences in their chemical structure or antigenic constitution. There can be no doubt that the same factors are responsible for differences between biological races of protozoa.

### Infection and Resistance

#### Effect of Parasite on Host

The entozoic protozoa of man are usually divided into pathogenic and non-pathogenic forms, the former producing recognizable diseases, varying in severity and frequently terminating fatally,



while the latter are commensals, which do not cause any discomfort to the host. However, these definitions have only a relative value and cannot be accepted unreservedly, especially in the case of the pathogenic protozoa. Most of the human intestinal amœbæ and flagellates normally behave as harmless commensals, and even the disease-producing forms do not invariably manifest their pathogenicity, for under certain conditions of the hostal environment they do not provoke any symptoms of disease but lead an apparently commensal existence. Thus, *Entamœba histolytica* frequently occurs in the lumen of the intestine where it lives as a commensal without doing any damage to the tissues, whereas in other cases it invades the intestinal wall or migrates to the liver, causing amœbic dysentery and liver abscess respectively.

The effect produced by entozoic protozoa upon man does not depend entirely upon the former but is determined by the interaction of the parasite and host, which are in constant conflict, the virulence or invasiveness of the entozoon struggling to overcome the resistance of the human host. When the opposing forces are well balanced, the parasite and host live in apparent harmony, the former leading a commensal existence without damaging the latter. But when the resistance of the host fails, the aggressive power or virulence of the parasite remains unchecked, with the result that it causes sufficient injury to provoke disease in the host. Even commensal protozoa which have never been incriminated in the production of human disease, have occasionally been known to behave like true parasites. Thus, there is on record a case of infection with *Iodamœba bütschlii*, in which the amœba—normally a commensal inhabiting the intestine—invaded various organs and tissues of the patient. Moreover, the change from parasitism to commensalism, and *vice versa*, is often accompanied by change of food habits. Thus, the tissue-invading form of *Entamœba histolytica* feeds on the red blood corpuscles of the host, whereas the lumen-dwelling form feeds on bacteria found in the intestinal contents. In the case mentioned above, the commensal *Iodamœba* acquired hæmatophagous habits.

In the examples just quoted the change of habit occurs in the human host, but in other cases the behaviour of the protozoa varies according to the host harbouring them. Thus, the pathogenic trypanosomes of man, *Trypanosoma rhodesiense* and *T. cruzi*, are

non-pathogenic to their supplementary or reservoir hosts, represented by antelopes and armadillos respectively. Similarly the ciliate, *Balantidium coli*, produces no pathological manifestations in the pig but may be pathogenic to man. Disease-producing protozoa are therefore in some cases only potentially pathogenic, their effect upon the host depending upon variations in the virulence of the parasite, on the one hand, and upon the conditions obtained in the host and its reactions to the activities of the parasite, on the other hand.

From the biological point of view, a state of equilibrium between host and parasite represents the normal condition, since it enables the parasite to complete its life-cycle and ensures the survival of its species by transfer to new hosts. However, when the infection provokes disease, especially with lethal results, the parasite is frequently unable to propagate its species. Thus, in amœbic dysentery *Entamœba histolytica* is discharged from the human body before it can encyst, in the form of active amœbæ, which perish rapidly without transmitting the infection to new human hosts. When the resistance of the host and the virulence of the parasite are well balanced and the two live in harmony, the host is said to be a CARRIER of the parasite. The carrier state, which is established either from the onset of the infection or after suppression of the clinical symptoms of the disease, provides optimum conditions for the survival of both parasite and host. Symptomless carriers are therefore of primary importance in the maintenance and dissemination of protozoal diseases in human communities. On the other hand, the diseased condition reflects an imperfect adjustment of the relations between the human host and the parasite, probably indicating that the association between them has only recently been established. From this point of view, those animals in which a parasite produces no symptoms and which act as its carriers, can be regarded as the original and natural hosts of the given parasite. Thus, antelopes and armadillos are probably the original hosts of *Trypanosoma rhodesiense* and *T. cruzi* respectively, from which man subsequently acquired his infection and which continue to act as reservoirs.

We can now consider more closely the effect of the parasite on the human host in protozoal diseases. The pathological action of the protozoa upon the human organism may be due to a number of

causes, among which the most important are (a) *mechanical injury*, (b) *abstraction of food-substances*, and (c) *chemical effect*. The effect produced by a given parasite may be due to one or another of these mechanisms, acting singly or in combination. Thus, invasion of the intestinal wall by the dysentery amœba, *Entamoeba histolytica*, is effected with the help of a proteolytic enzyme (cytolysin) which destroys the histological elements, enabling the parasite to penetrate deep into the tissues with the production of the characteristic ulceration. Here the effect is both chemical and mechanical. The lesions produced in the bowel by *Balantidium* affords another example of mechanical action. In the case of intracellular protozoa there is considerable destruction of the elements harbouring the parasites. Thus, the activities of the malaria parasites lead to a large-scale destruction of the erythrocytes, resulting in anæmia, while the febrile symptoms may be due to the release of toxins. In the Malignant Tertian form of malaria (*Plasmodium falciparum*) some of the most serious clinical manifestations are due to mechanical blocking of the capillaries in vital organs by the developing parasites. The cardiac symptoms characteristic of Chagas' disease are due to mechanical and functional damage resulting from the proliferation of *Trypanosoma cruzi* in the heart-muscle. Muscles are also directly injured by the Sarcosporidia. The pathological effects in Kala-Azar are brought about by invasion of the macrophages by *Leishmania donovani*, which results in blockade of the reticulo-endothelial system, accompanied by progressive anæmia due to involvement of the bone-marrow. In some protozoal diseases—especially in Toxoplasmosis (*Toxoplasma*), as well as in Chagas' disease and Sleeping Sickness—parasitic invasion of the central nervous system produces various cerebral symptoms. In Sleeping Sickness some of the clinical symptoms are probably due to consumption of blood sugar by the trypanosomes (*Trypanosoma gambiense* and *T. rhodesiense*), which impairs the glycogenic functions of the liver, while other pathological manifestations are thought to be due to toxins (trypanotoxin), released after death of the parasites. Though the question regarding the pathogenicity of *Giardia* is still unsettled, in view of the fact that this flagellate inhabits the duodenum it is conceivable that it deprives the human host of some of the food intended for his own consumption, on account of which *Giardia* has been aptly called a "food-robber."

The foregoing account gives only a general idea of the mechanisms responsible for various manifestations of disease produced in the human host by the pathogenic protozoa. The behaviour of the parasites in different protozoal diseases will be dealt with more fully in the systematic description given in Part II.

### Effect of Host upon Parasite

**Nature of Immunity.**—The infection of human beings by entozoic protozoa is determined by the ability of the parasites to overcome the resistance, or immunity, of the host, which is manifested by various defence reactions to the presence and activities of the parasites. There are two main types of immunity, **NATURAL** and **ACQUIRED**. Natural immunity is an inherent property of the host, producing an immediate response to the introduction of the parasite, while acquired immunity develops gradually in the course of an established infection. Among the factors responsible for immunity some are static, others dynamic. The former depend upon the physiological conditions of the hostal environment, which may be antagonistic to the parasite, while the latter are represented by elements of the host's organism, which are actively engaged in combating the parasite. All these factors are barriers which parasites must overcome before they can set up an infection in the host. Unfortunately, our knowledge of the mechanisms by which the host opposes the establishment of protozoal infections is very limited. In general, the natural host provides the parasite with optimum physiological conditions, such as temperature, food, etc., to which the latter has become adapted in the course of evolution. Thus, the human entozoic protozoa are usually able to set up an infection in man, owing to the fact that the human organism provides them with a suitable environment and because they are equipped to withstand the defence mechanisms of the host. On the other hand, foreign protozoa are prevented from establishing themselves in man owing to their inability to overcome the natural resistance, or immunity, of the host, which may be due either to the unsuitability of the static physiological conditions of the hostal environment or to active opposition on the part of the dynamic defence forces of the human body. Host-restriction on the part of parasites is one of the manifestations of natural immunity. While these factors are most effective against infection with foreign parasites, under certain

conditions they may also protect the host from infection with its natural entozoic protozoa.

**Mechanism of Immunity.**—Among the static mechanisms of defence may be mentioned the character of the digestive juices of the host and the host's diet. Thus, infection with human intestinal protozoa depends on their ability to escape from the cysts, which are digested or softened by the action of the digestive juices, while in the case of foreign protozoa the digestive juices are ineffective. It is known, for instance, that the oocysts of fish coccidia (*Eimeria*), when ingested by man, pass unchanged through the alimentary canal and are discharged with the faeces. It has also been demonstrated that variations in the host's diet may have a profound effect on the course of parasitic infections. Thus, the ciliate, *Balantidium coli*, which is common to pig and man, thrives best in the intestine of the pig, where it finds abundant and suitable food in the form of starch. However, human infections with this parasite are relatively rare, probably because conditions in the human bowel are not favourable to its existence, owing to the scarcity of starch. As a rule, intestinal protozoa are adversely affected by a high protein diet, whereas a carbohydrate diet favours their survival.

The effect of the static physico-chemical conditions of the hostal environment in preventing and controlling infection is especially marked in the case of the intestinal protozoa, whereas the defence mechanism against the blood- and tissue-inhabiting protozoa, in both natural and acquired immunity, is more dynamic, being based on a direct attack upon the parasites by phagocytic cells of the host's tissues and by substances known as antibodies, which are found chiefly in the plasma of the blood. These two elements represent the cellular and humoral mechanisms of defence respectively. Though present in normal persons and contributing to their natural resistance on first exposure to entozoic protozoa, the effect of these defence mechanisms increases considerably in the course of an infection and is responsible for the state of acquired immunity, which leads either to the destruction or suppression of the parasites, and protects the host against reinfection with the same species of parasite.

The cellular factors of immunity are represented by connective-tissue and blood cells, which are endowed with the power of phagocytosis. These cells, generally called phagocytes, actively ingest

and destroy the microorganisms which find their way into the host's body. Among these cells, the polymorphonuclear leucocytes, or heterophils, and the macrophages play the most important rôle. The polymorphonuclear cells are found chiefly in the blood, while the macrophages are derived from fixed and wandering cells of the reticulo-endothelial system or from lymphocytes, by direct transformation of the latter. Among the different organs of the human body those richest in elements of the reticulo-endothelial system, like the spleen, lymph glands, liver and bone-marrow, are the chief sources of phagocytes. In the course of an infection the phagocytes are stimulated to increased proliferation, with the result that the organs in question usually become considerably enlarged.

The humoral factors of immunity are represented by substances, known as ANTIBODIES, which appear in the blood serum as a reaction to the introduction of foreign proteins into the body of the host. The substances capable of stimulating the production of antibodies are known as ANTIGENS; they may be soluble foreign proteins or complete cells, including various microorganisms. The function of the antibodies is to combine with the antigens in such a way as to neutralize them or render them inactive, thereby protecting the host from any harmful effect on their part. This result may be achieved in different ways: (a) the antibody may sensitize the antigen and render it more vulnerable to the action of phagocytes (*opsonification*); (b) the antibody may bring about the death and disintegration of microorganisms (*lysis*); or (c) the antibody may neutralize the biological action of toxic antigens (*antitoxic effect*). The presence in the body of an antigen stimulates the formation of an antibody which may have any of the above effects, according to the nature of the antigen. A distinction is made between *immune antibodies*, formed in response to an infection with microorganisms, and *natural antibodies*, which are sometimes present in the blood of a normal host. Serum containing antibodies is known as antiserum.

It is thought that antibodies are products of metabolism of cells of the reticulo-endothelial system, which are secreted into the surrounding body fluids and tissues. Since both the cellular and humoral mechanisms of immunity are associated with macrophages, there is no fundamental difference between the two phenomena. In the case of phagocytosis the host-cell acts as a whole, while in the case of antigen-antibody reactions the active substance is a product

released from the host-cell, which may act upon the parasites directly or indirectly, by sensitizing them to phagocytosis. One of the most important attributes of antigen-antibody reactions is their *specificity*: each antigen stimulates the production of an antibody which differs from antibodies to any other antigens and combines only with homologous or closely related antigens. Hence, the immune response to infection with any particular microorganism is specific, though closely related species or races of microorganisms contain, in addition to *specific* antigens peculiar to each of them individually, common or *group* antigens, which are also capable of reacting with the corresponding antibodies.

It has already been noted that biological races of entozoic protozoa are analogous to intraspecific "types" among bacteria, the distinction between which resolves itself into differences in the chemical composition of their antigens, manifested by the specificity of their immunological reactions. Although antigenic analysis has only recently been applied to protozoa, the available data indicate that in the protozoa the difference between biological races is determined by the same factors as in bacteria and is likewise of a chemical nature.

Both the cellular and humoral factors contribute in various degrees and combinations to the development of immunity in the host. As a rule, the natural resistance at the beginning of an infection is non-specific, the parasites being kept in check by the phagocytes and/or natural antibodies. But as the infection progresses the parasitic antigens gradually bring about a specific immune response. The degree of immunity acquired in the course of an infection with different microorganisms varies considerably and is generally dependent on peculiarities of their antigenic constitution, which cannot be discussed here. In general, however, it may be said that the antigenicity of human entozoic protozoa is of a low grade, as compared with that of most pathogenic bacteria. On account of this the protozoa are incapable of stimulating the production of potent antibodies. Moreover, their antigens are unstable and subject to variation, as a result of which some protozoal species readily give rise to immunologically distinct strains and biological races. These factors determine some of the peculiarities of acquired immunity in protozoal infections of man.

The effect of immunity is twofold: on the one hand, it serves

to check an existing infection, by keeping the numbers of parasites at a low level; on the other hand, it protects the host against reinfection with the same species or strain of parasite. The type of immunity acquired in protozoal infections differs considerably from that typically observed in bacterial diseases. In the latter the high-grade antigenicity of the parasites usually elicits a powerful immune response on the part of the host, which may result in the complete destruction of the pathogenic organisms and protect the host against subsequent reinfection for variable periods of time after recovery. This type of resistance is known as **RESIDUAL IMMUNITY**. In protozoal diseases, especially in those due to blood- and tissue-inhabiting parasites (in malaria, trypanosomiasis and leishmaniasis), stimulation by parasites with a low-grade antigenicity produces a relatively feeble immune response, which is not strong enough to eliminate all the parasites but prevents them from multiplying excessively, with the result that the infection becomes latent and is maintained by a small number of surviving parasites. Owing to the slight immune response in this type of immunity, its persistence depends on continuous stimulation, which is exerted by the surviving parasites and is reinforced by repeated reinfections. On elimination of the parasites the resistance usually falls rapidly, leaving no residual immunity, but in some protozoal diseases it may afford protection against reinfection. This type of resistance, which depends on the presence of parasites, is sometimes called **CONCOMITANT OR TOLERANCE IMMUNITY** but is now more generally known as **PREMUNITION**. Thus, the main difference between the two types of acquired resistance is that residual immunity succeeds the infection, while concomitant immunity, or premunition, accompanies the infection. Premunition is characteristic of infections with protozoa of the blood and reticulo-endothelial system (leishmaniasis, trypanosomes and malaria parasites).

Very little is known regarding immunity to infection with intestinal protozoa. Those which live in the lumen of the bowel, without damaging the host's tissues, do not seem to be able to stimulate an immune response, with the result that the host can be readily reinfected. In the case of the tissue-invading *Entamoeba histolytica* there appears to be a marked response, detectable by serological tests.

A more detailed description of the mechanism and development



of immunity in some protozoal infections will be given in Part II.

**Vaccination.**—In bacterial and virus diseases immunity to infection can be produced artificially by inoculation, or vaccination, of human beings with killed or living microorganisms. The immune bodies produced in response to vaccination behave as in naturally acquired immunity, protecting the host against infection with the homologous parasites. The only protozoal disease in which vaccination is effective is cutaneous leishmaniasis, whereas in other protozoal diseases all attempts at immunization have been unsuccessful. This failure is due, on the one hand, to the inability of the protozoal antigens to elicit sufficiently strong and lasting immune response; and, on the other hand, to the existence within the same species of parasite of immunologically distinct strains and races, which are moreover liable to give rise to further variants. It would, therefore, be practically impossible to prepare a vaccine sufficiently polyvalent to protect the host against all the strains of a given parasite which are likely to be encountered.

**Serological Tests.**—Diagnosis of parasitic infections can be made by detecting the presence of antibodies in the serum or tissues of the host. Such serological tests are based on the fact that antibodies, when brought into contact with homologous antigens, will produce one of the characteristic reactions *in vitro*. Though of greatest practical value in bacteriology, these tests proved to be very disappointing in the diagnosis of protozoal diseases, owing to the peculiarities of the immune response which have already been discussed.

## ACTION OF DRUGS UPON PROTOZOA

Though the treatment of protozoal diseases is outside the scope of this book, this subject cannot be dismissed without giving a brief account of the principles of chemotherapy, by which is understood the treatment of parasitic infections with chemical compounds. In the past the approach to chemotherapy was purely empirical, the discovery of effective drugs being the result of "trial and error," but during the last quarter of a century its study has been placed on a more rational theoretical basis. It now seeks to correlate the chemical structure of drugs with their specific effect upon the parasites, which is attributed to combinations between chemical groups of the drug and chemical groups in the parasite. However,

our knowledge of these interactions is still very incomplete, for, while the study of the chemical structure and affinities of the drugs has advanced considerably, little is known about the reactions which go on within the parasites.

Though the exact mechanism of drug action upon the protozoa has not been fully determined, an approach has been made to an understanding of the main principles involved. A drug, to be effective, must have physical and chemical properties which enable it to reach the parasite within the body of the host. It must then be absorbed by the parasite in sufficient concentration to be lethal. Furthermore, since all chemotherapeutic agents are toxic to both the parasite and the host, i.e. they possess both parasitotropic and organotropic properties, the most suitable drugs are those which combine a maximum parasitotropic effect with a minimum of organotropic effect, i.e. which can destroy the parasites with the least harm to the host.

The action of a drug upon a parasite depends upon the presence on the former of active groups which combine with some chemical groups (chemoreceptors) in the parasite. The chemoreceptors are most probably situated upon enzymes and intermediate substances which play an important part in the metabolic processes of the parasite. The variety of chemical reactions taking place within the body of the latter are visualized as a series of enzymatic processes, in the course of which materials taken in from the outside are dealt with by a succession of different enzymes. By combining or competing with one or other of these, the drug interferes with the nutrition, respiration or reproduction of the parasite, and ultimately brings about its death. In the case of trypanosomes it has been shown that the effect of arsenicals is due to their combination with the thiol ( $-SH$ ) groups, which are connected with the hydrogenation processes of the parasites.

Various cytological changes have been observed in some protozoa (e.g. malaria parasites and trypanosomes) as the result of drug action but there is no clear evidence to distinguish a specific effect of a drug from the usual degenerative processes which occur in any dying cell. The chemotherapeutic action of a drug is also influenced by the chemical changes it undergoes in the body of the host before it reaches the parasite. Chemotherapeutic agents are often highly specific in their action, being as a rule fully effective only against

one species of parasite, though related species are sometimes also susceptible.

Among the numerous drugs employed in the treatment of protozoal diseases we shall briefly refer to the mode of action of some of the most important ones.

In amœbiasis alkaloids of the emetine series have a specific effect upon *Entamœba histolytica*, which is manifested both *in vitro* and in the treatment of amœbic dysentery. The drug probably reaches the parasites present in the ulcers through the host's tissues and acts upon them directly. As regards the amœbæ which may be present in the lumen of the intestine, it is possible that they are beyond the reach of the drug and therefore unaffected by emetine treatment.

The chief drugs used in the treatment of African trypanosomiasis or Sleeping Sickness, include suramin ( $\approx$  "Bayer 205") and tryparsamide. The toxic action of "Bayer 205" may be due to its inhibiting effect upon the carbohydrate metabolism of the trypanosomes, the impairment of which renders them more susceptible to phagocytosis. Tryparsamide is a pentavalent arsenical, which has no trypanocidal effect *in vitro*, but *in vivo* it penetrates into the host's tissues, where it is reduced to a trivalent form which is therapeutically active. Its effect is due to combination with the —SH groups present in the trypanosomes, resulting in an interference with the metabolic processes of the parasites. The arsenicals which are effective against the trypanosomes of Sleeping Sickness have no action upon the trypanosome causing American trypanosomiasis, or Chagas' disease.

The action of antimonials, which are used in the treatment of Kala-Azar, has points of resemblance to that of arsenicals, for the pentavalent antimonial must be reduced to trivalent form before it affects the leishmanias. It has been suggested that the effect of antimonials is to stimulate the cellular defence mechanism of the host, but this is improbable in view of the fact that the leishmanias actually inhabit elements of the reticulo-endothelial system which normally act as phagocytes. It would, therefore, seem that the parasites must be weakened in some way before they can be destroyed by the phagocytes.

The anti-malarial drugs, quinine, mepacrine ( $=$  atabrin) and paludrine, are thought to have a direct action upon the malaria

parasites, by interfering with their metabolism. It should be noted, however, that the anti-malarial drugs do not act upon all stages in the life-cycle of the parasite. Thus, all the above-named preparations kill the asexual stages in the blood but have no effect upon the sexual forms or upon the sporozoites, pamaquin (= plasmoquin) being the only known drug with a specific action upon the gametocytes. Paludrine, but not quinine, has an action against the presumptive tissue phase of *Plasmodium falciparum*.

In conclusion, mention should also be made of the phenomenon known as DRUG-FASTNESS, which has been more fully studied in trypanosomiasis. When a host is treated with doses which are too small to cure the disease, there may be temporary disappearance of the trypanosomes with a subsequent relapse of the infection. In such cases renewed treatment has no effect upon the relapse trypanosomes, which have become resistant to the drug, giving rise to a strain in which the resistance is hereditary. Administration of sub-curative doses of drugs is responsible for the artificial creation of drug-resistant strains of microorganisms in a number of infectious diseases. There is reason to believe that this phenomenon depends on the capacity of protozoa and other microorganisms to undergo spontaneous variation, or mutation, with the production of variants, including drug-resistant forms. The drug exercises a selective action upon the parasites, by killing all the susceptible members of a mixed population and leaving the resistant ones to reproduce themselves.

## GEOGRAPHICAL DISTRIBUTION OF PROTOZOAL DISEASES

The geographical distribution of parasitic diseases is determined by the presence in a given locality of the causative organism itself, of its host or hosts; and by various factors in the external environment.

The distribution of human protozoal infections varies according to whether the causative organisms are transmitted from man to man directly or through the intervention of an intermediate host. Since in the former case man is the only host involved, the entozoic parasites have accompanied him to all the inhabited parts of the world, their distribution, like that of the human host, being cosmopolitan. Thus, infections with intestinal protozoa occur wherever human beings are found, ranging from the polar regions through countries with a temperate climate, to the equator. The incidence

of intestinal protozoal infections in different countries varies considerably but this variation is due rather to the state of sanitation among the population than to climatic conditions. Insanitary conditions of life account for the fact that infections with *Entamoeba histolytica* are generally more prevalent in warm countries, though under similar conditions the incidence of infection in some temperate countries and even in the Arctic region may reach comparable figures (see Table 3). However, the influence of climatic conditions upon the course of amœbiasis, which is the only intestinal protozoal disease of any importance, cannot be excluded. As a rule, the severity of this disease is much greater in the tropics, owing to the harmful effect of the hot climate, unsuitable diet and other factors, which tend to lower the resistance of the host. Thus, although the geographical distribution of *E. histolytica* coincides with that of its human host, its incidence in different localities is influenced by the cultural level of the community, while the clinical manifestations of the infection are indirectly dependent on climatic conditions.

As regards the heteroxenous protozoa, their geographical distribution depends not only on the human host but also on the bionomics of the intermediate hosts, the distribution of which is more or less limited. In general, the distribution of insect-borne protozoal diseases coincides with that of their vectors, provided other local conditions favour transmission of the infection. Thus, the distribution of Sleeping Sickness is restricted to the intertropical belt of Africa, to which the vectors, represented by tsetse-flies (*Glossina* spp.), are confined, while the distribution of Chagas' disease coincides with the distribution of the Reduviid bugs, which act as vectors, in South and Central America. The different types of leishmaniasis (Kala-Azar, Oriental Sore) are widely distributed in the warm parts of Asia, Europe and America, wherever the vectors, represented by sandflies (*Phlebotomus* spp.), are found. Though the Anopheline mosquitos, which are the vectors of malaria, are almost cosmopolitan, the prevalence of this disease does not strictly coincide with the actual distribution of these insects. This is due to the fact that under natural conditions only certain species of *Anopheles* act as efficient vectors, owing to differences in breeding places, food preferences and the degree of contact with human beings. Moreover, climatic conditions, affecting both the mosquito and the parasite, also influence the distribution of malaria. Thus,

malaria parasites are incapable of developing in the intermediate host at temperatures below  $15^{\circ}\text{C}$ ., the optimum conditions for the transmission of the disease being at temperatures between  $20$  and  $30^{\circ}\text{C}$ . ( $68$ – $86^{\circ}\text{F}$ .) and at a mean humidity of 63 per cent. These factors limit the distribution of malaria to regions lying roughly between  $45^{\circ}\text{N}$ . and  $30^{\circ}\text{S}$ . latitude but in some countries extending to  $60^{\circ}\text{N}$ . and  $20^{\circ}\text{S}$ ., where the temperature is suitable for the development of the parasites, and to altitudes not exceeding 6,000 feet above sea level, where mosquitos do not breed. Furthermore, each of the four species of *Plasmodium* has its own area of distribution, in some places overlapping that of other species, in others independent (see Chapter 12).

There is considerable variation in the prevalence of protozoal diseases in different parts of their area of distribution, due to local factors. The disease may be constantly present among the population of a given locality, when it is said to be ENDEMIC. When its incidence is very high and the population has acquired partial immunity or premunition to the infection, as the result of years of exposure, the disease is said to be HYPERENDEMIC. In the case of diseases of lower animals, including mammalian reservoirs of human infection, the corresponding terms are ENZOOTIC and HYPER-ENZOOTIC. When the disease produces severe outbreaks locally or on introduction into a new community, it is called EPIDEMIC in man, and EPIZOOTIC in other animals.

ZOONoses, diseases common to man and some of the lower mammals, in which the latter serve as reservoirs of human infection, present other problems. Typical zoonoses have a restricted or focal distribution. Those affecting wild animals are usually confined to foci amid natural surroundings, where the infection circulates by insect-transmission from host to host, independently of man. Zoonoses involving domestic animals are present in foci which are usually in close contact with man. In diseases of the first type contact between the reservoir host and man is usually accidental: human beings temporarily entering such natural foci expose themselves to attacks by the vector, and may become infected, or they may settle down to live in a zone where such foci exist, with the result that the disease becomes permanently established in the human community. In diseases of the second type contact between the reservoir host and man is in some cases unlimited, affecting various

clements of the population, while in others it may be restricted to certain groups of people, the disease being in effect occupational. Examples of diseases common to man and wild mammals are provided by a form of Oriental Sore affecting gerbils in the deserts of Central Asia and by Chagas' disease affecting armadillos and some other animals. Zoonoses involving domestic mammals are represented, on the one hand, by the Mediterranean form of Kala-Azar which is prevalent among dogs; on the other hand, by Balantidiosis of pigs, which affects chiefly persons engaged in handling pigs (farmers, swineherds, slaughterers).

Protozoal infections can also be disseminated beyond their natural boundaries by the introduction of their vectors or infected persons into new localities, where the diseases may occur sporadically or establish themselves permanently under suitable conditions. Favourable conditions for the introduction of diseases into new areas are created by migrations of the people, which may occur under peaceful conditions but are particularly common during wars, when movements of troops, accompanied by evacuation or removal of the population, may result in the dissemination of diseases from one place to another. Thus, the civil war in Russia resulted in the spread of malaria to the extreme north of the country, where the presence of adequate vectors enabled the disease to persist for several years. It is also thought that Malignant Tertian malaria was originally introduced into the New World with slaves from Africa. While the full effects of the second World War cannot yet be fully assessed, it has already resulted in the introduction of malaria and amœbiasis into various countries by returning soldiers. Since in most of the protozoal diseases immunity is strain-specific, localities where a given disease is already endemic may be affected by the introduction of foreign strains of the parasite in the same degree as localities which have previously been exempt. The facilities for the dissemination of protozoal infections have increased considerably by the speed of modern transport by land, sea and air.

All these conditions have provided increasing opportunities for the introduction of protozoal infections, which are generally regarded as "tropical diseases," into countries with a moderate climate. The medical practitioner in such countries must therefore be prepared at least to recognize those protozoal diseases which in the past were dealt with by specialists in tropical medicine.

# PART II

## SYSTEMATIC ACCOUNT OF THE PROTOZOA

### SECTION A

#### PROTOZOA OF THE ALIMENTARY AND GENITAL TRACTS

THE alimentary and genital tracts of man may be parasitized by 16 species of protozoa representing all the 4 classes of the sub-kingdom PROTOZOA.

TABLE 2  
PROTOZOA OF THE ALIMENTARY AND GENITAL TRACTS

CLASS	COLLECTIVE NAME	GENUS	SPECIES	HABITAT
RHIZOPODA	Amœba	<i>Entamœba</i>	<i>histolytica</i> <i>coli</i> <i>gingivalis</i>	intestine intestine mouth
		<i>Endolimax</i>	<i>nana</i>	intestine
		<i>Iodamœba</i>	<i>bütschlii</i>	intestine
		<i>Dientamœba</i>	<i>fragilis</i>	intestine
MASTIGOPHORA	Flagellates	<i>Embadomonas</i>	<i>intestinalis</i>	intestine
		<i>Chilomastix</i>	<i>mesnili</i>	intestine
		<i>Enteromonas</i>	<i>hominis</i>	intestine
		<i>Trichomonas</i>	<i>hominis</i> <i>tenax</i> <i>vaginalis</i>	intestine mouth vagina
		<i>Giardia</i>	<i>intestinalis</i>	intestine
SPOROZOA	Coccidia	<i>Isospora</i>	<i>belli</i> <i>hominis</i>	intestine intestine
CILIOPHORA	Ciliates	<i>Balantidium</i>	<i>coli</i>	intestine



Fifteen of these species inhabit the alimentary tract (2 in the mouth and 13 in the intestine) while only one occurs in the genital tract. The arrangement of these protozoa in systematic order is given in Table 2.

The majority of intestinal protozoa cause no harm to man and lead a commensal life, feeding on the contents of the gut. Some, namely *Entamæba histolytica* and *Balantidium coli*, are highly pathogenic, being capable of causing severe symptoms of disease, though in some cases their virulence is not manifested and they may also behave like commensals. Others, like *Giardia intestinalis* and *Isospora belli*, may provoke mild symptoms of disease, but their pathogenicity has not been conclusively proved. The genital flagellate, *Trichomonas vaginalis*, is in the same position. As regards the protozoa living in the mouth, *Entamæba gingivalis* and *Trichomonas tenax*, they have also been suspected of causing various disorders, but there is not sufficient evidence to support the accusation.

The life-cycle of the intestinal protozoa typically consists of two phases: an active, or trophic, stage, when they live in the bowel where they feed and multiply; and a resting, or encysted, stage which is discharged in the fæces and which serves for the dissemination of these protozoa. In the resting stage they are capable of surviving outside the human body for some time. Infection takes place by the mouth, if food or drink contaminated by the resistant form of the parasite are swallowed by a new host. In the alimentary canal the resting stage gives rise to the trophic form and the condition known as infection is initiated in the host. On the other hand, the oral and genital protozoa are transmitted to new hosts by direct contact.

## CHAPTER 4

### THE AMŒBÆ

THE amœbæ, which comprise some of the simplest forms of protozoa, belong to the order Amœbida of the class RHIZOPODA (see Chapter 2). The majority of amœbæ are free-living and aquatic, inhabiting fresh, brackish and salt water, or moist soil, but a large number are parasitic in the alimentary canal of various animals, including man.

All amœbæ have the following characteristics in common. Their body is naked, i.e. it is not covered by any form of shell or membrane, except when encysted; and when in a state of activity it has no fixed shape or orientation, so that it is impossible to define anterior and posterior ends or sides of the body (Figs. 4; 10; 12, a, e; 18, a, c). The cytoplasm is differentiated into a hyaline outer ectoplasm and an inner mass of vacuolated or granular endoplasm, containing the nucleus, food-vacuoles and other inclusions. Locomotion is effected by means of pseudopodia. These arise as cytoplasmic currents which flow in certain directions and cause the ectoplasm at these points to protrude in the form of lobe- or finger-like processes, the amœba progressing in the direction of the pseudopodia (Fig. 5, b). This type of movement, which is also observed in some other protozoa (e.g. malaria parasites), is known as amœboid. The amœbæ do not possess a special oral opening and ingestion of food takes place at any point of the body, the food-particles or microorganisms being engulfed by a pseudopodium. In the endoplasm the food-particles are surrounded by a drop of fluid to form food-vacuoles, within which they are gradually digested. The undigested excreta are cast out through the body-wall.

In the alimentary tract of man there are six species of amœbæ, five of which are true amœbæ, belonging to the class Rhizopoda (*Entamoeba histolytica*, *E. coli*, *E. gingivalis*, *Endolimax nana*, *Iodamoeba bütschlii*), while one represents the amœboid phase of an aberrant flagellate (*Dientamoeba fragilis*). Five of these amœbæ live in the intestine and one (*E. gingivalis*) in the mouth (Figs. 4, 10, 12).

In the majority of human amœbæ the cycle of development consists of two main stages, the active or free amœba (sometimes

described as the vegetative form) and the cyst. The active amœbæ are the trophic forms feeding and multiplying within the intestine and giving rise to the condition called infection, which may or may not produce symptoms of disease, according to the species concerned.

At certain periods of their life the amœbæ become rounded, cease to feed, expel all food-remnants from the body and become surrounded by a resistant membrane which protects them against desiccation and other unfavourable conditions outside the human body. In this state the amœbæ (and other protozoa) are said to be encysted and are known as cysts, which—in parasitic amœbæ—are voided in the fæces (Figs. 4, c-f; 10, d-g; 12, b-d, g).

The cysts are the only forms which remain viable outside the human body, whereas the active amœbæ soon die. The cysts are therefore the only stage capable of transmitting the infection from man to man. Unlike the cysts of some free-living protozoa which are formed occasionally as a protection against unfavourable conditions in their environment, the cysts of parasitic amœbæ represent an integral stage of their life-cycle. They serve not only to protect the enclosed amœbæ while these are outside the host but also to propagate their species. In the case of those amœbæ in which cysts have not been discovered (*Entamæba gingivalis*, *Dientamæba fragilis*) the method of transmission remains unsolved. Infection with intestinal amœbæ takes place through the mouth, by swallowing their cysts. When the cysts are ingested, they pass into the intestine, where they hatch and liberate the amœbæ. The amœbæ then reach their appropriate place in the bowel and start a new infection.

Thus, the life-cycle of those human intestinal amœbæ which produce cysts passes in two environments—the bowel and the outside medium. No secondary, intermediate host or vector is required, though other animals, e.g. insects, may help to disseminate the cysts.

The human amœbæ multiply by simple binary fission (Fig. 5, c, e) and no sexual processes, such as conjugation, are known to occur in them.

The differentiation of the genera and species of amœbæ is based primarily on the structure of the nucleus and cysts, while the appearance of the cytoplasm and the dimensions of these organisms

afford supplementary diagnostic characters. The most important differential characters of the human amœbæ will be found in Table 4, which follows the description of the separate species. Of the six species of amœbæ living in man only one, *Entamœba histolytica*, is pathogenic, the other five being harmless commensals.

## GENUS *ENTAMŒBA* CASAGRANDI & BARBAGALLO, 1895

(Not *Endamœba* Leidy, 1879).

The genus *Entamœba* comprises three species parasitic in man, *E. histolytica*, *E. coli* and *E. gingivalis* (Figs. 4, 10). Many other species of this genus occur in vertebrate animals of all classes. The chief character distinguishing the genus *Entamœba* from other genera of amœbæ is the nuclear structure. The nucleus (Fig. 1, A) is of the vesicular type, containing a relatively small karyosome which may be central or eccentric, and chromatin granules distributed uniformly or irregularly on the nuclear membrane, while in some species chromatin granules also occur in the space between the karyosome and the membrane.

### i. *ENTAMŒBA HISTOLYTICA* SCHAUDINN, 1903

Synonyms: *E. dysenteriae* (Councilman & Lafleur, 1891); *E. hartmanni* Prowazek, 1912; *E. dispar* Brumpt, 1925; *Endamœba histolytica* (Schaudinn, 1903).

Relation to Disease.—*Entamœba histolytica*, commonly known as the "dysentery amœba," is a pathogenic parasite of man, responsible for the condition known as AMŒBIASIS, manifestations of which vary from symptomless infection of the large intestine to acute amœbic dysentery. *E. histolytica* may also produce metastatic invasion of the liver and some other organs.

Geographical Distribution.—*E. histolytica* is cosmopolitan in its distribution, occurring in countries with temperate, subtropical and tropical climates throughout the world, extending beyond the Arctic circle in the north and to the Straits of Magellan in the south.

Habitat.—*E. histolytica* normally inhabits the large intestine of man, from the ileo-cæcal valve to the rectum, where it may be found in the lumen and/or within the wall of the gut.

## MORPHOLOGY AND LIFE-HISTORY

The active forms (or trophic amœbæ) of *E. histolytica* (Fig. 4, a ; 5, a, b), when rounded, measure from  $10\mu$  to  $40\mu$  in diameter, the dimensions varying according to the conditions under which they are living in the intestine of the host. When living as commensals in the lumen of the gut, as in carriers or in chronic infections, they measure from  $10\mu$  to  $20\mu$  in diameter (Fig. 5, a) and are sometimes described as "*minuta*" forms, but when they invade the intestinal wall they increase considerably in size, measuring from  $20\mu$  to  $40\mu$  (Figs. 4, a ; 5, b).

The endoplasm of *E. histolytica* is finely granular, whereas the ectoplasm is hyaline in appearance and well differentiated from the endoplasm. There is a single nucleus, measuring from  $4\mu$  to  $7\mu$  in diameter, according to the size of the amœba. When seen during life it is invisible or inconspicuous but when fixed and stained it has the following structure. The nuclear membrane is lined with a single layer of fine chromatin granules, usually of equal size, so that the nucleus appears to be outlined by a ring of minute beads, which form the so-called peripheral chromatin. In the centre of the nucleus lies a small karyosome, measuring about  $0.5\mu$ , which consists of a darkly staining granule of chromatin surrounded by a clear zone or halo. The achromatic network in the area between the karyosome and the membrane does not contain any chromatin. The central position of the karyosome, the thin ring of peripheral chromatin and the absence of chromatin granules between the karyosome and membrane are the chief nuclear characters distinguishing *E. histolytica* from the closely related *E. coli*, though in the nuclei of recently divided amœbæ of the former species the karyosome may be eccentric.

**Locomotion.**—One of the characteristic features of *E. histolytica* is its movements. When examined in a fresh faecal specimen, removed from the stool while it is still warm, or on a warm stage of the microscope, the amœba is extremely active. Its body pushes out a single long finger-like pseudopodium (Fig. 5, b) and seems to flow along at great speed. However, when cooled it remains stationary but changes shape all the time, owing to the formation of large blunt hyaline pseudopodia (Fig. 4, a). These consist of ectoplasm alone and are sharply demarcated from the endoplasm.

*E. histolytica* sometimes remains active in that way for hours. No other intestinal amœbæ reveal such activity.

**Nutrition.**—Like all amœbæ *E. histolytica* ingests solid food by means of its pseudopodia, while fluid food is absorbed through the surface of the body. The food-habits of this amœba are discussed more fully below.

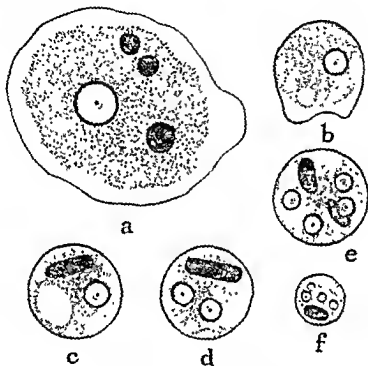


FIG. 4.—*Entamoeba histolytica*: THE DYSENTERY AMŒBA ( $\times 2,000$ ).  
(Adapted from Dobell and O'Connor, 1921.)

small race, with chromatoid body.

Reproduction takes place by binary fission (Fig. 5, c, c), in the course of which the nucleus divides by a form of mitosis with the formation of chromosomes (about 8) and a spindle, after which the cytoplasm becomes constricted into two equal portions which separate, giving rise to two daughter-amœbæ. In these the nuclei are reconstructed and assume the typical appearance of the resting stage, though the karyosome of recently divided amœbæ may be

more or less eccentric. Multiplication occurs both in the lumen and in the tissues of the intestine.

**Encystation.**—Encystation occurs in the lumen of the bowel. The active amœbæ divide, giving rise to smaller amœbæ, which expel all food-particles contained in the endoplasm and cease to feed. The resulting small amœbæ, which have a nucleus with a slightly eccentric karyosome and a clear cytoplasm, are known as PRECYSTIC FORMS (Figs. 4, b ; 5, f). They are somewhat smaller than the lumen-form ("*minuta*"), their size corresponding to that of the cysts to which they give rise. The precystic amœba rounds up and secretes around its body a thin membrane or capsule which forms the cyst-wall.

The CYSTS (Figs. 4, c-f ; 5, g-i ; Pl. I) are usually spherical, sometimes ovoid, and may be very slightly asymmetrical. They measure from  $5\mu$  to  $20\mu$  in diameter, according to race (Fig. 11). The cyst-wall, which can be seen in living specimens but is invisible in stained ones, consists of a single layer measuring about  $0.5\mu$  in thickness. The cysts are at first uninucleate (Figs. 4, c ; 5, g), the nucleus not differing in structure from that in the free amœba. In the course of further development the nucleus divides into two (Figs. 4, d ; 5, h), then each of these divides again, so that finally a 4-nucleate cyst is produced (Figs. 4, e ; 5, i ; Pl. I). As the nuclei increase in number they diminish in size but retain their typical structure, though frequently the peripheral chromatin granules in the bi- and quadri-nucleate cysts are more concentrated at one point of the membrane, producing a crescentic thickening at one side (Fig. 4, e). In unstained cysts the nuclei are invisible (Pl. I).

In addition to the nucleus, cysts commonly contain two cytoplasmic inclusions—CHROMATOID BODIES and GLYCOGEN. The chromatoid bodies usually have the appearance of refractile rods with rounded ends (Pl. I ; Figs. 4, c-f ; 5, g-i) or of irregular small bodies which stain deeply with chromatin stains (e.g. with hæmatoxylin). In the cytoplasm of the encysted amœba there also occurs a vacuole varying in size and filled with glycogen, as shown by its brown colour when treated with iodine solution (glycogen reaction) (Pl. I). These inclusions are sometimes revealed already in the precystic amœba. Both the chromatoids and glycogen represent reserve food material (protein and carbohydrate respectively). The number of chromatoids and the amount of glycogen

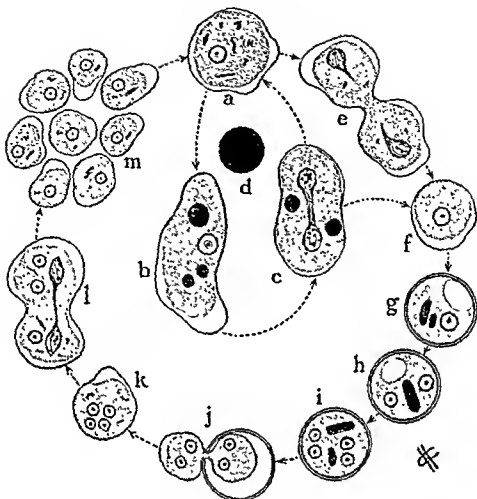


FIG. 5.—LIFE-CYCLE OF *Entamoeba histolytica* ( $\times 1,300$ ). (Original and adapted from Dobell, 1928.)

a, e-m. Development in lumen of intestine: a. Amœba with ingested bacteria ("minuta" form); e. Division of amœba; f. Precystic amœba; g, h, i: uni-, bi-, and quadri-nucleate cysts, with glycogen vacuole and chromatoid bodies; j. Excystation (hatching) of quadri-nucleate amœba; k-m. Metacystic stages: k. Excysted quadri-nucleate amœba; l. Division of metacystic amœba; m. Eight of metacystic amœba. b-c. Invading amœba with ingested amœba; d. Normal red blood corpuscle.

present in cysts varies but sometimes both elements are altogether absent. As a rule, the glycogen begins to disappear from the cysts by the time they reach the quadri-nucleate stage, while the chromatoids may persist longer, but ultimately they also disappear, both inclusions being used up as reserve food.



The cysts are voided in the faeces in all stages of development, but apparently the immature uni- and bi-nucleate ones are incapable of developing further outside the body, while the quadrinucleate cysts, representing the mature infective stage, remain viable in the stool for a considerable period of time and, if swallowed by man, initiate a new infection.

**Excystation.**—When the ripe 4-nucleate cysts are swallowed by a human being, they probably pass unchanged through the stomach and hatch in the small intestine. This is deduced from the fact that the cyst-wall is insoluble in gastric juice but is soluble in trypsin. The details of excystation (Fig. 5, j-m) have been established from observations on cultures of *E. histolytica*. The quadrinucleate amoeba enclosed within the cyst escapes through a minute pore in the wall. In the course of its subsequent development (metacystic stages) it goes through a series of nuclear and cytoplasmic divisions which result in the production of eight uninucleate amoebulae. These small amoebae pass into the large bowel, where they grow and are transformed into the large active amoebae already described. It has been shown, in experimental infections of dogs, that the cysts may hatch within  $4\frac{1}{2}$  hours after ingestion. Apparently they do not hatch in the intestine of the original host. The complete life-cycle of *E. histolytica* is shown in Fig. 5.

**Races.**—*E. histolytica* comprises at least two races which differ in the size of their cysts. One is a small race with cysts having a mean diameter of  $8\mu$  or  $7\mu$  (Fig. 4, f), the other is a large race with a mean diameter of  $12\mu$  or  $11\mu$  (Fig. 4, c-e), the two measurements referring to cysts measured in living and stained specimens respectively (Figs. 6, 11). In practice a diameter of  $10\mu$  can be taken as a dividing line between the two races, cysts measuring less probably belonging to the small race, while those measuring more probably belong to the large race. Except for their dimensions, the two races are morphologically indistinguishable but it is thought that they may also differ in certain biological properties (see below). Some authors regard the small race as a distinct species, *E. hartmanni*. However, in view of an overlap in their dimensions, there is more justification for regarding them as subspecies rather than species. If this course is adopted, the small race should bear the name *E. histolytica hartmanni*, while the large race becomes *E. histolytica histolytica* (see Chapter 2).

**Atypical Forms.**—The structure of the active amœbæ and cysts of *E. histolytica* described is that seen in well preserved and stained preparations of normal individuals. However, there frequently occur degenerating forms, especially among amœbæ from stale stools. In such forms the structure, especially that of the nucleus, shows various abnormalities, such as irregular clumping of the peripheral chromatin, disintegration of the karyosome, vacuolation

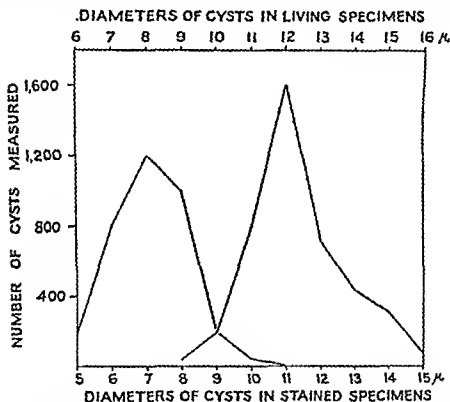


FIG. 6.—DISTRIBUTION OF CYST MEASUREMENTS IN THE SMALL AND LARGE RACES OF *Entamoeba histolytica*. (Adapted from Saper, Hakansson and Louttit, 1942.)

of the cytoplasm, and so on. Sometimes cysts develop abnormally and may contain 8 nuclei (supernucleate cysts). Such aberrant forms are liable to be misinterpreted. Thus, a cyst with 8 nuclei might be mistaken for *E. coli*.

**Cultivation.**—*E. histolytica* can be readily grown at 37° C. in artificial media (for a description of one of these see Chapter 19). A sample of feces containing amœbæ or their cysts is usually inoculated into the medium together with the accompanying

intestinal flora, or washed and suitably treated cysts can be inoculated together with selected species of bacteria. Living bacteria are indispensable for the growth of *E. histolytica* in culture, certain species being more favourable than others. In culture the amœbæ feed on bacteria and on granules of starch which is added to the medium, as well as on dissolved matter contained in it. The cultural forms of amœbæ are smaller than the tissue-invading forms in man, and correspond more closely to the commensal forms ("minuta" phase) living in the lumen of the bowel. The active amœbæ can usually be induced to encyst and excyst *in vitro* by a change in their diet. Thus, if a culture grown with starch is subinoculated into starch-free medium, then grown in several subcultures in similar medium for a week, and afterwards transferred again to a medium containing starch, the amœbæ will encyst in the last culture, provided suitable bacteria are also present. In order to induce excystation the culture containing cysts is removed from the incubator and kept for some time at room temperature or in a refrigerator, after which it is subinoculated in fresh starch-containing medium, in which the cysts will hatch. However, it has been demonstrated that cysts are also capable of hatching at body-temperature, without previous cooling.

### HOST-PARASITE RELATIONSHIP

It has already been stated that the natural habitat of *E. histolytica* in man is the large intestine. In the great majority of cases the infection produces no visible signs of disease, while in others there are definite clinical symptoms, from minor intestinal disorders to typical amœbic dysentery.

#### Harmless Infections

In a great number of cases the infection is limited to the lumen of the bowel, the amœbæ living on the surface of the mucous membrane and among the contents of the gut, where they feed on bacteria, starch granules and possibly on faecal debris. In stained preparations of amœbæ from the faeces of such cases bacteria can be clearly seen within the food-vacuoles (Fig. 5, a). When grown in cultures, as well as in natural and experimental infections of macaque monkeys and rats, *E. histolytica* reveals the same food-habits as it does when living in the lumen of the human gut. Owing

to the relatively poor diet on which the lumen-dwelling amœbæ (the so-called *minuta* forms) subsist, they do not grow to a large size, their measurements being from  $10\mu$  to  $20\mu$  in diameter. The amœbæ multiply and from time to time (according to some observers—periodically) pass into the precystic stage and encyst, the cysts being evacuated together with the fæces. When the bowels are functioning normally and formed stools are produced, the presence of cysts in them may be the only indication of an amœbic infection in the host, but when the stools are loose, medium-sized amœbæ, some of which contain ingested bacteria, and small precystic amœbæ may be mingled with the cysts. The amœba inhabiting the lumen of the gut and producing cysts apparently lives in harmony with its host as a commensal and can be regarded as the normal trophic or vegetative form of *E. histolytica* (Fig. 5, a), while the host himself is to all intents and purposes a healthy carrier of the parasite.

### Pathological Effect

However, the host-parasite relationship in amœbiasis is not always so harmonious, for *E. histolytica* is potentially pathogenic, being capable of developing virulent properties which are manifested by invasion of the intestinal wall by the lumen-inhabiting amœba. In such cases the amœbæ attach themselves to the mucous epithelium and secrete a cytolytic ferment (cytolysin) which destroys the host-cells. The amœbæ gradually erode the surface of the intestinal wall and penetrate actively into the deeper tissues, first breaking through the muscularis mucosæ, then into the submucosa, where they spread laterally, undermining the mucous membrane and in severe cases even penetrating the muscular coats. In this way an amœbic ulcer is produced (Fig. 7), which is typically flask-shaped with a relatively narrow canal leading through the mucosa into the submucosa, where it becomes dilated. As the amœbæ invade the tissues they are continuously multiplying and feeding on the cytolysed tissue and on red blood corpuscles liberated by extravasation of blood into the lesion. The amœbæ are found chiefly at the periphery and at the base of the ulcer (Fig. 8), in contact with the healthy tissue into which they are gradually working their way, while the cavity of the ulcer is filled with necrotic tissue. The extent of damage done to the intestinal wall varies considerably: in many cases there is only a superficial erosion of the mucosa, in

others there are "pin-point" microscopic lesions, and in others again typical deep ulcers varying in size. When the contents of an ulcer are extruded into the lumen of the gut they carry with them some of the amœbæ, which attack other parts of the mucous membrane and may be passed in the fæces. These tissue-invading amœbæ are larger than the lumen forms, their measurements varying from  $20\mu$  to  $40\mu$ . Their endoplasm may contain ingested erythrocytes in various numbers and in different stages of digestion, giving the amœba a characteristic appearance which is of diagnostic

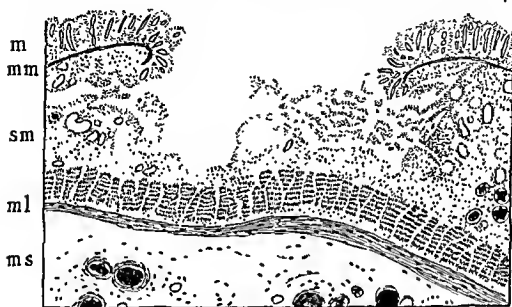


FIG. 7.—SECTION ACROSS AN AMEBIC ULCER OF THE COLON.  
(Adapted from Bartlett, 1917).

m. Mucosa ; mm. Muscularis mucosæ ; sm. Submucosa ; ml. Muscular layers ; ms. Mesentery.

value (Figs. 4, a ; 5, b). The amœbæ do not encyst in the ulcers. The cysts are produced in the lumen of the gut either by the tissue-inhabiting amœbæ which emerge from the ulcers or by those amœbæ which had remained in the lumen (*minuta* forms) without invading the tissues. Some authors believe that the amœbæ living in the lumen are the only forms capable of encysting and propagating their species.

The entire life-history of *E. histolytica* is represented graphically in Fig. 5.

The lesions are commonly found in the cæcum and adjoining parts of the colon (cæcal area), and in the rectal area, including the

rectum itself and the sigmoid flexure, but in severe cases the entire large bowel may be involved (Fig. 9).

As regards clinical manifestations of amœbiasis, there may be every gradation from symptomless infection to acute disease, the severity of symptoms not being necessarily correlated with the degree of damage done to the intestinal tissues.

**Symptomless Infections.**—In a large proportion of cases the superficial lesions of the mucous membrane and shallow ulcers are

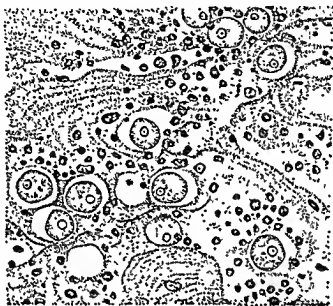


FIG. 8.—*Entamoeba histolytica* IN THE SUBMUCOSA OF THE HUMAN LARGE INTESTINE IN AMŒBIC DYSENTERY ( $\times 500$ ). (Adapted from Wenyon, 1926.)

continually healed and there is no visible evidence of a diseased condition. In other cases well-developed ulcers have been discovered *post mortem* in persons who had never revealed any signs of disease during their life. In infections of this type and in those considered above, in which the amœbæ live commensally without invading the tissues, an equilibrium is established between parasite and host, enabling the amœbæ to complete their development normally, with the production of cysts which are passed in the stools. Such persons, having symptomless infections with *E. histolytica*, are known as "CARRIERS." When their stools are solid and formed, they usually pass only cysts, and the finding of these is the sole

evidence of an amœbic infection in carriers. Here it may be mentioned that some clinicians refer to such cases as "cyst-carriers" and speak of the effect of drugs upon "cysts," ignoring the fact that the cysts in the fæces can only be derived from active amœbæ present in the gut of the patient. If the stools are loose or semi-solid the lumen-dwelling amœbæ (*minuta* and precystic forms) may also be found in the fæces. There are two types of carriers: CONTACT carriers, who have never shown any evidence of illness due to their infection with *E. histolytica*, and CONVALESCENT carriers, who had previously had clinical symptoms of disease but have recovered, though still harbouring amœbæ in their gut. The contact carriers include both persons in whom the amœbæ live as commensals without invading the gut wall as described above, and persons in whom intestinal lesions are present without any outward symptoms of disease. However, these two kinds of contact carriers are indistinguishable during life. Carriers represent the vast majority of cases of amœbiasis: it has been reckoned that 90 per cent. of persons infected with *E. histolytica* do not show any symptoms of disease.

**Clinical Manifestations.**—In the clinical forms of amœbiasis the symptoms vary from minor intestinal disorders to dysentery, and they are always associated with more or less extensive ulceration of the large bowel. In the milder cases the infected individuals may suffer from diarrhœa and will pass in their stools precystic amœbæ together with cysts, and sometimes also large amœbæ, containing ingested erythrocytes, which have found their way out of the intestinal ulcers. The stools in diarrhœa may contain mucus but no blood. When the condition becomes more severe, owing to extensive ulceration and destruction of the capillaries, the intestinal wall becomes necrotic, and blood and mucus escape into the lumen of the gut, carrying with them numerous tissue-inhabiting amœbæ which often contain red blood corpuscles (Figs. 4, a; 5, b). All these elements are passed in the stools, and the patient is said to be suffering from typical amœbic dysentery. The disease may be acute or chronic. In chronic cases periods of quiescence or latent periods, in the course of which lumen-dwelling amœbæ (*minuta* and precystic forms: Figs. 5, a; 4, b) and cysts are passed in the stools, may alternate with relapses, when clinical symptoms reappear. Infection with *E. histolytica* appears to be of unlimited duration and may persist for the rest of life.

## Food-Habits

From the foregoing account it is seen that the food-habits of *E. histolytica* vary according to its mode of existence in the bowel. In clinical cases with ulceration of the intestinal wall, when the amœbæ invade the tissues, they ingest red blood corpuscles and other tissue-elements of the host. In symptomless infections—

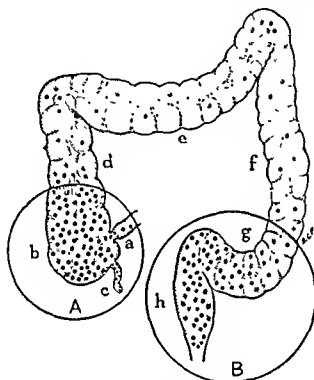


FIG. 9.—THE LARGE BOWEL IN AMŒBIASIS. (After Faust, 1943: *Trans. Stud. Coll. Physicians, Philadelphia.*)

Distribution of lesions, showing their preponderance in cecal area (A) and rectal area (B). a. Ileum; b. Cæcum; c. Appendix; d. Ascending; e. Transverse; f. Descending, and g. sigmoid colon; h. Rectum.

which constitute the majority of cases—as well as in chronic amœbiasis, the lumen-dwelling amœba feeds on bacteria and other contents of the gut. In addition to formed particles *E. histolytica* also absorbs fluid food saprozoically. The dimensions of the amœbæ appear to be correlated with their mode of nutrition: they are smallest when feeding on bacteria, larger when starch is added to their diet (as in cultures), and largest of all when feeding on erythrocytes and other tissue-elements of the host. The commensal



form of *E. histolytica* is not commonly observed, since persons with a symptomless infection either escape detection or their stools, when examined, contain only cysts.

The commensal habits of *E. histolytica* in some forms of symptomless infection were noted by a number of observers, but their views have not been generally accepted, many parasitologists and most clinicians maintaining that *E. histolytica* is an obligatory tissue-parasite which invariably invades the gut-wall, where it feeds on the host's tissues. However, recent investigations on the behaviour of this amœba in cultures and in lower mammals (see below) as well as direct observations in man lend support to the view that it is also capable of living in the human gut as a commensal.

That *E. histolytica* is omnivorous is also evident from the fact that its food-habits can be changed at will. Thus when amœbæ, which have been living as commensals and feeding on bacteria in the intestine of a monkey, are transferred to a kitten, they invade its gut-wall and start feeding on red blood corpuscles, but if the amœbæ from an infected kitten are recovered in culture, they revert to their original food-habits and ingest bacteria.

### Incubation Period

The incubation period of amœbiasis varies. In experimental infections it has been found that cysts first appear in the stools from 1 to 44 days (average 9 days) after inoculation. In some cases of amœbic dysentery the first symptoms can be observed in from 20 to 95 days after ingestion of cysts.

### Metastatic Infections

One of the most serious complications of intestinal amœbiasis is a metastatic invasion of the liver. The amœbæ may find their way into the capillaries or lymphatic vessels of the intestinal wall, whence they are drawn into the portal circulation and carried to the liver. In the liver they first give rise to hepatitis and later to one or more abscesses, usually in the right lobe. The amœbæ attack the hepatic cells which are cytolysed and absorbed, the liver tissue undergoing necrosis with the formation of pus filling the cavity of the abscess. In typical cases of amœbic liver abscess its contents are bacteriologically sterile. Within the liver abscess the amœbæ

are found along the wall, in contact with healthy tissue which they are continually invading, thus increasing the size of the abscess.

*E. histolytica* sometimes also invades the lung, producing pulmonary abscesses. It is thought that they gain access to the lung from a liver abscess. The amœbæ may be found in material expectorated after rupture of an abscess into the lung. *E. histolytica* has also been known to attack the brain with the formation of cerebral abscesses, but such cases are very rare. Occasionally the amœbæ produce ulceration of the skin in the anal region, around a colostomy wound, or at the site of drainage of a liver abscess. Such infections of the skin are due to a direct extension of the visceral infection. The secondary sites of infection (liver, lung, brain, etc.) are inhabited only by the large active amœba of the same type as that found in the ulcers of the large bowel. In these sites the amœbæ feed and multiply but do not encyst.

### Pathogenesis

*E. histolytica* is a pathogenic parasite, in the sense that it is endowed with the power of invading the tissues of its host, producing lesions and clinical symptoms of disease. However, in the great majority of cases the amœba does not manifest any evidence of virulence, for in about 90 per cent. of persons harbouring *E. histolytica* the infection is symptomless. In some of these carriers the amœba lives as a harmless commensal, without invading the tissues of the gut, though it is conceivable that it might invade the tissues and produce lesions at some period of the infection. But even when the gut-wall is invaded, in most cases the reparative powers of the host are capable of restricting the damage, with the result that no clinical symptoms are revealed. In a minority of cases the host's defence is broken and the disease develops unhampered.

We are still in ignorance regarding the factors responsible for the development of the disease.

**Host-Resistance.**—According to some investigators the course of infection with *E. histolytica* depends mainly upon variations in the susceptibility of the human host. Thus, experimental infections carried out with human volunteers have demonstrated that one and the same strain of *E. histolytica*, when inoculated into a number

of men, produced in the majority a symptomless infection, in a few typical amœbic dysentery and in individual cases no infection at all.

**Strains of the Parasite.**—Other authorities attribute the differences in the manifestations of amœbiasis to the existence in different localities of races or strains of *E. histolytica* differing in virulence. In support of this view it is pointed out that, although the incidence of infection in countries with a temperate or cold climate is comparable to that in hot countries, manifestations of disease, including dysentery, occur more frequently in the tropics, the infections in temperate regions being usually symptomless. Observations made in Germany have shown that in the interior of the country only carriers of *E. histolytica* are found, whereas cases of dysentery are restricted to the ports, into which virulent strains have been introduced from the tropics. It is also known that Europeans develop clinical symptoms of amœbiasis more readily in the tropics than in their own country, where they are equally exposed to the danger of infection. It is possible that they are adapted and therefore resistant to home strains of the amœbæ but susceptible to infection with more virulent foreign strains. On the other hand, the greater severity of the disease in hot countries is attributable to lower resistance on the part of the host due to an unfavourable climate, unsuitable diet and similar causes.

*E. dispar*.—It may also be noted that an attempt has been made to explain the variation in virulence by assuming the existence of two distinct species of amœbæ, one pathogenic, the other non-pathogenic. According to this view, clinical cases with invasion of the tissues are due to *E. histolytica* proper, whereas symptomless infections in carriers are attributed to another species, for which the name *E. dispar* was proposed, though it is admitted that these two "species" are morphologically indistinguishable. However, there are no grounds for accepting this interpretation.

**Small Race of *E. histolytica*.**—There seems to be a general agreement of opinion that in human infections clinical symptoms are never associated with the presence of amœbæ belonging to the small race, the cysts of which measure on the average 7-8 $\mu$  in diameter (Fig. 4, f). On this account, it is believed that the small amœbæ are not pathogenic to man. However, this point has not been definitely settled and stands in need of confirmation.

**Intestinal Flora.**—Some observers believe that invasion of the intestinal tissues by the lumen-inhabiting form of *E. histolytica* is influenced by the presence in the intestinal flora of certain bacteria, such as dysentery bacilli. According to this view, these bacteria are the first to damage the walls of the gut, thereby preparing the way for the amœbæ, which can then attack the tissues. Though based on some experimental evidence this hypothesis stands in need of further investigation. There can be no doubt, however, that bacterial infections may be responsible for some complicating symptoms in amœbic dysentery.

It is probable that any or all of the factors mentioned above may influence the course of amœbic infection, which is determined by the relation of the virulence of *E. histolytica* to the resistance of the host, the virulence of the parasite increasing as the host-resistance is lowered.

### Immunity

Very little is known regarding immunity to infection with *E. histolytica*. There is evidence of the existence of natural immunity in certain individuals, who either fail to acquire an infection or in whom the infection is symptomless, as in carriers. However, there does not appear to be any acquired immunity, for the attack does not protect against reinfection with *E. histolytica* and a person cured of amœbic infection is liable to be reinfected.

### Incidence

Surveys conducted since the world war of 1914-1918 have shown that in various countries and in different climates, a fairly high percentage of the indigenous population examined is infected with *E. histolytica*. The great majority of these persons (estimated at 90 per cent.) do not show any symptoms of disease and represent carriers, some of them being healthy carriers, others carriers with cryptic lesions, and others again convalescent carriers. The incidence of amœbic infection in a number of countries is shown in Table 3.

From Table 3 it is evident that, on the whole, the incidence of amœbiasis in warm countries is not appreciably higher than that in colder countries; it is actually determined not so much by climatic conditions as by the sanitary conditions under which the people live.

TABLE 3

INCIDENCE OF INFECTION WITH *ENTAMOEBA HISTOLYTICA*

COUNTRY	PERCENTAGE INFECTED	COUNTRY	PERCENTAGE INFECTED
Britain . . .	2.9- 9.7	India . . .	19.9
France . . .	5.0- 6.5	Malaya . . .	14.5-29.5
Holland . . .	16.0-28.0	Java . . .	23.0-27.8
Italy . . .	38.6	China . . .	3.0-20.0
Spain . . .	21.0	Manchuria . . .	15.9-24.4
Sweden . . .	1.5- 9.4	Egypt . . .	5.3-11.5
Finland . . .	8.8	U.S.A. . .	1.4-55.5
Soviet Union :		Panama . . .	2.8-72.7
Kola Peninsula . . .	6.3-60.6	Mexico . . .	6.0-85.0
Leningrad . . .	14.2-25.3	Venezuela . . .	6.8-42.9
Moscow . . .	3.3	Brazil . . .	10.4-47.5
Caucasus . . .	12.5-34.0	Argentina . . .	8.7-33.0
Central Asia . . .	2.4-43.3	Cuba . . .	1.2-30.8
		Australia . . .	4.6

## Infection in Lower Mammals

Considerable light has been thrown on the course of amœbic infection in man by studies on natural and experimental infections of some lower animals.

**Monkeys.**—Natural infections with *E. histolytica* are common in monkeys, most of the observations having been made on macaques (genus *Macacus* = *Silenus*). The identity of the human and simian strains of *E. histolytica* was demonstrated by cross-infections which have shown that the amœbæ of monkeys and man are interchangeable. The amœbic infections in monkeys afford a good example of perfect adaptation between parasite and host. The amœbæ—both in natural and experimental infections of monkeys—are of medium size, corresponding to the “*minuta*” form in man, and usually live as harmless commensals in the lumen of the gut of their host, feeding on bacteria and faecal debris. The infection is persistent, having been observed up to 16 months. As a rule the amœbæ in monkeys do not invade the tissues of the intestinal wall or ingest red blood corpuscles, nor do they produce any symptoms of disease. However, like the human strains of *E. histolytica*, they are pathogenic, since they are capable of producing typical amœbic

dysentery when inoculated into kittens, and intestinal disorder when their cysts are fed to human volunteers. It is conceivable that in countries where there is contact between monkeys and man, these animals may serve as a reservoir of human infection, and *vice versa*.

**Rats.**—Wild rats sometimes harbour an amœba indistinguishable from *E. histolytica*, and since there is some evidence that the infected animals have been in contact with human cases of amœbiasis, it is thought that they derive their infection from man. This view is supported by the fact that rats are susceptible to infection with human strains of *E. histolytica*. Experimental infection of rats, induced by feeding cysts, or inoculating them intracæcally with active amœbæ of human origin, is at present widely employed for the study of amœbicidal properties of drugs. In infected rats the amœbæ are found chiefly in the cæcum, where they live either as harmless commensals, feeding on bacteria and starch contained in the cæcal contents and forming cysts, or they invade the intestinal wall, producing typical lesions and ingesting red blood corpuscles. The course of infection in rats thus shows the same variation as in man, though the damage done to the intestinal wall is not so extensive and symptoms of dysentery do not develop. While monkeys, like man, can be regarded as natural hosts of *E. histolytica*, it would seem that spontaneously infected rats derive their infection from human sources. It is possible that under suitable conditions rats might serve as a vehicle for the dissemination of cysts and thus be of some epidemiological importance in the transmission of *E. histolytica* to man.

**Dogs.**—Natural infections with *E. histolytica* have also been reported for dogs, and there is reason to believe that, like rats, they acquire the infection from human beings. They are also susceptible to experimental infection with human strains of the amœba. The infection, which is localized chiefly in the cæcum, may run a symptomless course, but frequently the amœbæ invade the tissues, producing lesions with symptoms of chronic or acute amœbiasis, including dysentery. As a rule, only active amœbæ are passed in the stools.

**Cats.**—Among the animals used for experimental infection with *E. histolytica* young kittens are the most susceptible. When inoculated with cysts *per os* or with active amœbæ *per rectum*, they usually develop symptoms of acute amœbic dysentery, with extensive

ulceration of the gut-wall. The amœbæ ingest red blood corpuscles and are passed in fluid stools with blood and mucus. They appear to be unable to encyst in the cat, nor have natural infections with *E. histolytica* been recorded from this animal. Cats are very suitable animals for studying amœbic dysentery and they are commonly used for testing the virulence of human and animal strains of *E. histolytica*.

### TRANSMISSION

From the point of view of transmission, carriers—both of the contact and convalescent types—are of the greatest importance, owing to the fact that they pass mature quadrinucleate cysts, which are the only stage of *E. histolytica* capable of producing infection in man. Patients suffering from amœbic dysentery and passing only active amœbæ in the stools are not infective to others, since ingestion of such forms does not give rise to infection.

Cysts appear to be produced cyclically, periods in which they are found in the stools in large numbers alternating with periods in which none or small numbers are present. It has been calculated that carriers of *E. histolytica* discharge anything up to 6,000,000 cysts per gramme of faeces, the total output per day being from 330 thousand to 45 million.

### Viability

Transmission of *E. histolytica* is closely connected with its vitality under different conditions.

### Active Amœbæ

The active amœbæ do not live very long outside the human body, the time of their survival in the stool depending on the surrounding temperature. Thus, at room temperature (22–25° C.) they can survive for from 6 to 16 hours, but in a refrigerator at 5° C. they remain viable from 2 to 3 days; on the other hand, if the faeces are kept in an incubator at 37° C., the amœbæ usually live only from 2 to 5 hours, probably owing to the inhibiting effect of excessive growth of bacteria. In cultures kept at 37° C. the amœbæ have been known to live up to 5 weeks without subinoculation, while at room temperature cultures sometimes survive up to 3 days. When treated with N/20 (about 0.2 per cent.) HCl amœbæ die in a few minutes. Treatment with hydrochloric acid for 2 hours

of material containing both active amœbæ and their cysts is used for the separation of the latter from the former, since the cysts are not affected by the acid.

### Cysts

As regards the cysts, the outside world is their natural environment in the period of transition from one host to another, and they are accordingly adapted to withstand prolonged exposure to external conditions.

**Effect of Temperature.**—In a stool kept at room temperature they may survive for at least 2 weeks and in a refrigerator for about 2 months, while in water or in culture they may remain viable up to 5 weeks at room temperature. In this connexion it is interesting to note that viable cysts of *E. histolytica* have been recovered from sewage. High temperatures are harmful to cysts and kill them in a short time, their thermal death point being 50° C. At body-temperature they do not survive in fæces more than a few days. Desiccation is rapidly fatal to cysts, therefore infection cannot be carried in dust. By soiling the hands with infected fæces it has been demonstrated that cysts die in from 5 to 10 minutes when dried on the hands and in 45 minutes under the finger-nails.

**Effect of Chemicals.**—The cysts of *E. histolytica* are resistant to the action of a number of disinfectants. A 2 per cent. solution of potassium permanganate does not kill them for several days, and chlorine in amounts usually employed for water purification has no effect upon them; a 1 : 2500 solution of bichloride of mercury as well as a 1 per cent. solution of either carbolic acid or lysol kills them in 30 minutes, while a 1 : 250 aqueous solution of cresol kills them in 5–10 minutes and a 1 : 20 solution immediately.

**Disinfection.**—In the absence of facilities for the proper disposal of fæces, they can be disinfected and the cysts of *E. histolytica* destroyed by the addition of 1 : 200 aqueous solution of cresol until the mixture has a fluid consistency, the time of exposure being 15 minutes.

**Treatment of Water.**—The cysts of *E. histolytica* can be removed from water by filtration and killed by boiling the water. Both the standard processes employed for the purification of the water supply, such as precipitation and rapid filtration through sand beds, and filtration through filters used for domestic purposes, are



effective in eliminating the cysts. The large size of the cysts ( $5-20\mu$ ) prevents their passage through filtering material with small pores.

**Flies and Cockroaches.**—It has been demonstrated experimentally, by feeding house-flies (*Musca domestica*) on infected human faeces, that the cysts of *E. histolytica* can survive in the gut of the fly, remaining viable in the droppings, 48 hours after the infective feed. Both the amœbæ and their cysts also retain their viability in the vomitus of flies up to 17 and 64 minutes respectively. Under natural conditions cysts of *E. histolytica* have been recovered from the gut of flies which were caught in houses inhabited by infected persons. An examination of "wild" flies in Mesopotamia (Iraq), during the first World War, revealed in the gut of 0.3 per cent. of the flies human faeces with cysts of the dysentery amœba. If it is taken into consideration that a fly starts defecating a few minutes after it has fed, it can readily be understood what an important factor the house-fly represents in the spread of amœbic infection. Similar observations on cockroaches have also incriminated these insects in the dissemination of cysts of *E. histolytica*.

### Methods of Transmission

Infection with *E. histolytica* is acquired by swallowing the cysts discharged in the faeces of infected persons, among whom carriers are of primary importance. For infection to take place human food or drink must be contaminated with such faeces, or there must be direct contact with infected faeces. This may occur under the following conditions:—

(1) Through pollution of the water supply, which can be effected by people defecating into the streams and rivers, as commonly practised in the East Indies; by using ground water (e.g. from wells) contaminated with cysts washed out from faeces deposited on the surface of the soil or from primitive pit-latrines; or, under civilized conditions, in the case of defective plumbing, when communication is established between the sewage and water-piping. Infections spread by this method are known as WATER-BORNE.

(2) Through contamination of vegetables grown in soils manured with human excrement, e.g. in China, Japan and some other countries.

(3) Through contamination of food and drink by FOOD-HANDLERS who happen to be carriers of the infection.

(4) Through the agency of house-flies which had previously been in contact with or fed on human faeces and then carried the cysts in their droppings or deposited them with their vomitus. Infections spread by this method are known as FLY-BORNE.

(5) Through direct contact from man to man, owing to lack of personal hygiene, e.g. in mental hospitals, children's homes, etc.

(6) Through some of the lower mammals infected with *E. histolytica* and passing cysts. Thus, it is possible that in some tropical countries monkeys might act as reservoir hosts, while in other countries rats might serve as vehicles of the infection.

**Prevention.**—Under modern conditions it is usually not difficult to ensure a safe water-supply, even under field conditions. Thus, the efficiency of the water-supply in the North African campaign of the recent war was demonstrated by the absence of any water-borne disease. In most cases, infection with *E. histolytica* is either fly-borne or derived from human carriers.

The methods of dissemination suggest the following precautions against amœbic infection: (1) Protection of food from flies; (2) Filtration or boiling of drinking water; (3) Avoidance of uncooked vegetables (cysts of *E. histolytica* are killed in 30 minutes at 45° C. and in 5 minutes at 68° C.); (4) Examination of food-handlers and elimination of amœbic carriers among them; (5) Sanitation in general.

## DIAGNOSIS

The parasitological diagnosis of amœbiasis is based entirely on the finding of *E. histolytica* or its cysts in the faeces or tissues of the patient. The stages discharged in the faeces depend on the condition of the patient and on the consistency of the stool at the time of examination. In normal formed stools passed by persons showing no symptoms or only mild symptoms of intestinal disorder usually only cysts in different stages of development (1-, 2- or 4-nucleate) are encountered. Loose semi-solid stools may contain medium (*minuta*-forms) or small (pre-cystic forms) amœbæ in addition to cysts. In diarrhœic stools there may be pre-cystic amœbæ and occasional large amœbæ with ingested erythrocytes, as well as cysts. In amœbic dysentery the liquid stools contain almost exclusively the large tissue-invading amœbæ, many of which enclose ingested red blood corpuscles.

The stool is first examined microscopically in fresh faecal preparations. It has been estimated that a single examination reveals only 25 per cent. of infected cases; therefore, it is important that the stools of suspected persons should be examined repeatedly, on different days. When absent in natural stools, amœbæ can sometimes be demonstrated after administration of a saline purgative. In the case of cysts, the chances of discovery are doubled (50 per cent.) if the cysts are concentrated by the zinc sulphate flotation method. Cultivation is a useful supplementary method of diagnosis but unreliable if employed alone, for in some cases there is no growth in culture even when faeces known to contain amœbæ or cysts are inoculated in the medium. The best results are obtained by combining all these diagnostic methods: examination of faecal preparations, concentration and culture. To these may be added sigmoidoscopy which is resorted to for the recovery of amœbæ in scrapings from ulcers, after all other methods have failed to reveal *E. histolytica*. In the case of liver or lung abscesses, an examination is made of material obtained by aspiration, in the former case, or of that expectorated by the patient, in the latter case. If the true nature of the amœbæ cannot be established in fresh preparations, they can be identified in permanent preparations of fixed and stained smears of faeces or other material.

The faeces should be examined as soon as possible after the stool has been passed. This is especially important in the case of active amœbæ, which rapidly degenerate, losing their motility and characteristic structure outside the human body. For this reason, material containing the amœboid forms should not be dispatched by post. However, wet-fixed smears of such material, made on coverslips and placed in 70 per cent. alcohol (see Chapter 18) can be preserved indefinitely and stained when required. If immediate examination is not practicable, e.g. when a faecal specimen has to be sent away for examination, it should be kept cold: under these conditions the amœbæ remain recognizable for several hours, but die more rapidly if kept warm. These precautions are not essential when dealing with cysts, which remain viable in the faeces outside the human body for at least a fortnight. Faecal specimens containing cysts of *E. histolytica* can, therefore, be sent by post or kept in the laboratory or, better, in a refrigerator, to be examined when convenient.

The general technique of and procedure in faecal examination, as well as special methods employed in the parasitological diagnosis of amœbiasis, including cultivation, are fully described in Chapters 16 and 19.

The identification of *E. histolytica* in the faeces, especially in unstained preparations, cannot be made with certainty without a knowledge of the appearance and behaviour of the four species of non-pathogenic amœbæ which may inhabit the human intestine. It will, therefore, be necessary first to give a description of these amœbæ and then return to the differential diagnosis of *E. histolytica*.

## ii. *ENTAMŒBA COLI* (GRASSI, 1879)

Relation to Disease.—*Entamæba coli* is not pathogenic to man.

### MORPHOLOGY AND LIFE-HISTORY

Of the four non-pathogenic human intestinal amœbæ *Entamæba coli* is the most important one, because it is the only species showing a close resemblance to the pathogenic *E. histolytica*, with which it may be confused in some stages.

*E. coli* has a world-wide distribution and may occur in up to 30 per cent. of the population; like *E. histolytica*, it inhabits the large intestine.

The active or trophic amœba (Fig. 10, b) is of about the same size as that of *E. histolytica*, being usually from  $20\mu$  to  $30\mu$  in diameter when rounded. Its nucleus has essentially the same structure, differing in the following particulars: the karyosome, which is surrounded by a halo, is larger and typically eccentric in position, while the peripheral chromatin has the appearance of a thicker ring, being composed of coarser granules than in *E. histolytica*; moreover in *E. coli* the achromatic network between the karyosome and the nuclear membrane also contains chromatin granules (Fig. 1, A). The ectoplasm is not so pronounced as in *E. histolytica* and there is no sharp demarcation between it and the endoplasm. Owing to its coarser structure the nucleus of *E. coli* can be discerned in the living amœba.

In contrast to *E. histolytica*, the movements of *E. coli*, when examined in a faecal preparation at room temperature, are very sluggish, though when observed at body-temperature it may be

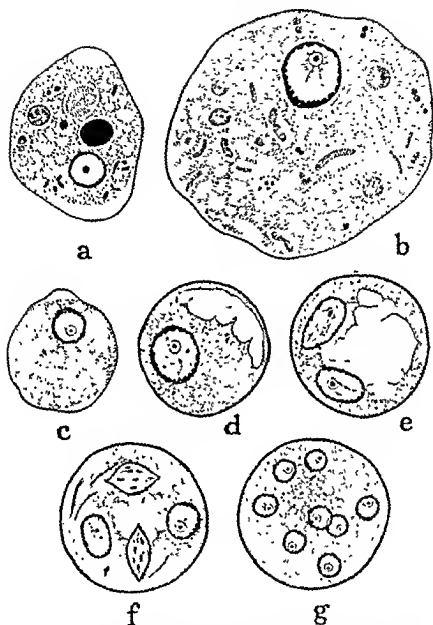


FIG. 10.—NON-PATHOGENIC ENTAMOEBAE ( $\times 2,000$ ). (Adapted from Dobell, 1919, and Dobell and O'Connor, 1921.)

a. *Entamoeba gingivalis*, from human mouth; b-g. *E. coli*, from human intestine: b. Active (trophic) amoeba with ingested bacteria, etc.; c. Precystic amoeba; d-g. Cysts: d. 1-nucleate with glycogen vacuole; e. 2-nucleate with large glycogen vacuole; f. 4-nucleate (2 nuclei dividing), with filamentous chromatoids; g. 8-nucleate (mature).

quite active. When moving the amoeba does not advance but changes its shape without forming large finger-like pseudopodia which are characteristic of *E. histolytica*.

**Nutrition.**—Another important feature in which *E. coli* differs from *E. histolytica* is its food-habits. *E. coli* never invades the tissues of the large intestine but lives in the lumen, where it feeds on various microorganisms and fragments present in the contents of the gut. Its endoplasm accordingly contains food-vacuoles filled with bacteria, yeasts, starch grains, vegetable particles and even protozoa (Fig. 10, b). The normal food of *E. coli* is thus the same as that of the commensal form of *E. histolytica* (Fig. 5, a). *E. coli* does not normally feed on red blood corpuscles, therefore in practice the presence in the stool of amœbæ with ingested erythrocytes can be safely attributed to infection with *E. histolytica*. It should be noted, however, that on one or two occasions human cases have been reported with *E. coli* containing red blood corpuscles, and it has been demonstrated that in culture this amœba can sometimes be induced to ingest these cells. On the other hand, the differentiation of *E. coli* from the commensal (*minuta*) form of *E. histolytica*, which has similar food-habits, is very difficult and should be based on the morphological characters, which can be seen best in properly fixed and stained preparations. Degenerate specimens of *E. coli* show various abnormalities of structure and may be indistinguishable from similar forms of *E. histolytica*.

**Reproduction.**—The method of reproduction in *E. coli* is by binary fission, as in *E. histolytica*.

**Encystation.**—Before encysting the active forms of *E. coli* divide, giving rise to smaller amœbæ. These expel all food inclusions, become rounded and are known as precystic amœbæ (Fig. 10, c), which are practically indistinguishable from the corresponding forms of *E. histolytica*. The cysts of *E. coli* may measure from  $10\mu$  to  $30\mu$  in diameter, the most frequently occurring dimensions being from  $15\mu$  to  $20\mu$  (Fig. 11). When newly formed, the cyst of *E. coli* is uninucleate (Fig. 10, d). By successive nuclear divisions it becomes first binucleate (Fig. 10, e), then quadrinucleate (Fig. 10, f) and finally octonucleate (Fig. 10, g). The 8-nucleate cyst is the mature infective stage of *E. coli*, but occasionally supernucleate cysts with 16 nuclei are produced. The nuclei in cysts have the same appearance as in the active amœbæ and, unlike those in cysts of *E. histolytica*, they are visible in living specimens (Pl. 1). The only stage in which cysts of *E. coli* might be confused with cysts of *E. histolytica* is the 4-nucleate one. However, in *E. coli* this stage

is passed through so rapidly that such cysts are very rarely encountered in stools. Moreover, one or more nuclei in the 4-nucleate cysts usually show signs of active division, being elongated or spindle-shaped (Fig. 10, f). In practice, therefore, the confusion with mature cysts of *E. histolytica* is not likely to arise.

As in the case of *E. histolytica*, the cysts of *E. coli* may contain glycogen which in this species is more abundant in the earlier stages, as shown by its dark-brown colour and sharp outline when treated with iodine. In the 2-nucleate cyst (Fig. 10, e) the glycogen vacuole may be so large that it occupies the greater part of the body and displaces the nuclei to the periphery. Binucleate cysts of this type, with the nuclei at opposite sides, are characteristic of *E. coli*. However, while in *E. histolytica* the glycogen may persist in the 4-nucleate stage, in cysts of *E. coli* it usually disappears by the time they reach the 4-nucleate stage and is usually absent in the mature 8-nucleate cysts.

Chromatoid bodies are not so common in *E. coli* as they are in *E. histolytica*. When present, they appear in the form of splinters or filaments (Fig. 10, f), sometimes sheaves of these, and are sharply pointed at the ends.

Excystation.—The 8-nucleate cysts are discharged with the stools and must be swallowed by a human being to produce infection. They probably hatch in the small intestine, but this process is somewhat different from that in *E. histolytica*. At excystation the entire 8-nucleate amœba escapes from the cyst through a large opening in the cyst-wall. It then undergoes a series of irregular binary fissions of the cytoplasm—without nuclear divisions—as the result of which 8 small uninucleate amœbulæ are produced. The small amœbæ grow and give rise to the large active amœbæ in the large bowel.

Cultivation.—*E. coli* can be grown in the same media as *E. histolytica* but they are more difficult to cultivate, owing to their greater susceptibility to variation of temperature and of hydrogen-ion concentration.

Occurrence in Lower Mammals.—*E. coli* occurs naturally in some monkeys, the simian strain being transmissible to man. Whether or not these animals can serve as reservoirs of human infection is not known.

Size of Cysts in *E. coli* and *E. histolytica*

In concluding the description of *E. coli*, attention should be drawn to certain additional points in the differentiation of this species and *E. histolytica*. This is based not only on the morphological differences already mentioned but also on the relative dimensions of their cysts. It is sometimes stated that the cysts of *E. coli* are larger than those of *E. histolytica*. This statement is only partly true and is misleading unless qualified. As a matter of fact, the range of measurements of cysts of *E. histolytica* is from  $5\mu$  to  $20\mu$  in diameter, while that of *E. coli* is from  $10\mu$  to  $30\mu$ . From these figures it is evident that the dimensions of the cysts in these two

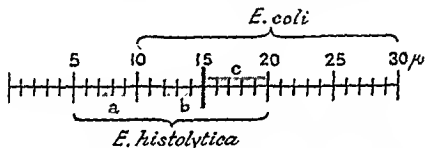


FIG. 11.—DIMENSIONS OF CYSTS OF ENTAMŒBÆ.

*Entamoeba histolytica*: range  $5\text{--}20\mu$  in diameter; a, b. Most frequent dimensions of cysts of small and large races respectively. *E. coli*: range  $10\text{--}30\mu$  in diameter; c. Most frequent dimensions.

species of amœbæ overlap, those measuring from  $10\mu$  to  $20\mu$  being common to both. However, measurements made in the course of numerous surveys by different observers have shown that the most common cysts of *E. histolytica* are those measuring from  $7\mu$  to  $9\mu$  (the small race) and those measuring from  $12\mu$  to  $15\mu$  (the large race), while in *E. coli* the cysts most frequently encountered measure from  $15\mu$  to  $20\mu$ . In other words,  $15\mu$  can be taken as the dividing line between these two species, cysts smaller than  $15\mu$  in all probability belonging to *E. histolytica*, those larger than  $15\mu$  probably representing *E. coli* (Fig. 11). Therefore, in actual practice it is safe to assume that cysts of *E. coli* are usually larger than those of *E. histolytica*, provided it is borne in mind that this distinction is only relatively true.



iii. *ENTAMOEBA GINGIVALIS* (GROS, 1849)

Relation to Disease.—*Entamoeba gingivalis* may be associated with pyorrhœa alveolaris, but its pathogenicity is doubtful.

## MORPHOLOGY AND LIFE-HISTORY

The third species of the genus *Entamoeba* occurring in man, *E. gingivalis* (Fig. 10, a), inhabits the oral cavity. *E. gingivalis* is similar in structure to its congeners, *E. histolytica* and *E. coli*. Its body, when rounded, measures from  $10\mu$  to  $20\mu$  in diameter. The ectoplasm and endoplasm of the oral amœba are clearly differentiated. It is more active than *E. coli*, producing short round pseudopodia.

The nucleus has the same appearance as in other entamœbæ; the karyosome is small and may be central or eccentric in position, while the peripheral chromatin granules lining the membrane are so close to each other as to form a continuous ring in optical section.

*E. gingivalis* does not produce cysts, its transmission probably being effected in the amœboid stage by direct contact, from mouth to mouth, e.g. when kissing, or by contamination of drinking vessels with infected saliva.

Nutrition.—The food-vacuoles contain bacteria and greenish granular inclusions, which represent the remains of ingested leucocytes and tissue cells, leucocytes apparently being eaten in preference to other elements. It is thought that *E. gingivalis* might also ingest red blood corpuscles.

Reproduction takes place by binary fission.

Viability.—*E. gingivalis* is remarkably resistant to changes in temperature: it can survive for 20 minutes at  $45^{\circ}\text{C}$ ., 48 hours at  $15^{\circ}\text{C}$ ., and 18 hours at freezing point. It is also more resistant to desiccation than the intestinal entamœbæ, and is tolerant to a wide range of hydrogen-ion concentrations (pH).

## HOST-PARASITE RELATIONSHIP

In the mouth *E. gingivalis* lives in the tartar and *materia alba* of the teeth, within carious teeth and in the pus pockets of pyorrhœa. Its distribution is probably world-wide with a fairly high incidence. As regards its pathogenicity, opinions are divided: some authorities believe it is a harmless commensal which acts as a scavenger of diseased tissue when pyorrhœic lesions are present, while others

regard it as the cause of pyorrhœa alveolaris. Though the incidence of amœbic infection corresponds to the degree of deterioration of the teeth, there is no evidence of any pathological action. Moreover, the number of parasites present is usually too small to account for any diseased condition of the buccal cavity.

iv. *ENDOLIMAX NANA* (WENYON & O'CONNOR, 1917)

Relation to Disease.—*Endolimax nana* is not pathogenic to man.

MORPHOLOGY AND LIFE-HISTORY

*Endolimax nana* is the smallest of the amœbæ inhabiting the large intestine of man. It is a harmless commensal, living in the lumen of the gut and feeding on various microorganisms present in the intestinal contents, but never invading the tissues. It is widely distributed throughout the world and has been reported in up to 26 per cent. of the population examined.

The size of the active amœba (Fig. 12, a), when rounded, varies from 6 to 12 $\mu$  in diameter, the average being about 8 $\mu$ . Thus, the larger forms fall within the range of the small forms of *E. histolytica* and *E. coli*, while the smaller forms of *Endolimax* are about as large as a human erythrocyte.

The movements of *E. nana* outside the human body are slow and soon cease.

*E. nana* can be readily differentiated from all the other human amœbæ by the peculiar structure of its nucleus. In the active amœba the nucleus, which is not easily seen in living specimens, has a large polymorphic karyosome, the appearance of which in different individuals varies considerably. It may consist of one more or less irregular mass of chromatin, usually eccentric, or it may consist of two or more lobes joined by strands. The nuclear membrane usually contains a few minute granules which may be connected with the karyosome by fine radial filaments. Outside the bowel the amœba soon degenerates and its nucleus then assumes the appearance of a signet-ring, owing to clumping of the karyosome at one pole.

Reproduction.—*E. nana* multiplies by binary fission with mitotic division of the nucleus in the course of which 10 chromosomes are produced.

**Encystation.**—Encystation is preceded by division of the amœba, which gives rise to precystic amœbæ measuring only about  $3.5\mu$  in diameter. Like the precystic forms of the entamœbæ, they do not

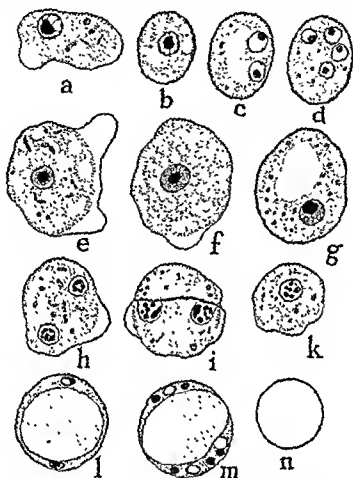


FIG. 12.—NON-PATHOGENIC AMŒBÆ AND *Blastocystis* ( $\times 2,000$ ).  
(Adapted from Dobell, 1940, 1943; Dobell and O'Connor, 1921; and Wenyon, 1926.)

a-d. *Endolimax nana*: a. Active amœba; b, c, d. Uninucleate, binucleate and quadrinucleate cysts. e-g. *Iodamoeba bütschlii*: e. Active amœba; f. Precystic amœba; g. Cyst. h-k. *Dientamoeba fragilis*: h, i. Binucleate amœbæ (in i showing chromosomes and centrodemesus); k. Uninucleate amœba resulting from division of binucleate form. l, m. *Blastocystis hominis*. n. Erythrocyte drawn to scale.

contain any food-vacuoles. The precystic amœba becomes rounded, secretes a cyst-wall and is thus transformed into a cyst. As a rule, the cysts are oval or spherical, measuring  $6-9\mu$  by  $5-7\mu$  (Fig. 12, b-d). They are at first uninucleate; the nucleus then divides successively and gives rise to two and finally to four nuclei, the 4-nucleate cyst

being the mature infective stage of *E. nana*. In the cysts the karyosome loses its polymorphism and usually appears as an eccentric globular mass. The immature (uni- and bi-nucleate) cysts may contain a glycogen vacuole, but as a rule the glycogen disappears before they reach the quadrinucleate stage. Most mature cysts, therefore, do not contain any glycogen. Chromatoid bodies do not occur in the cysts of *E. nana*.

**Excystation.**—The cysts are passed in the stools and are capable of surviving outside the body for 2 or 3 weeks at room temperature. When ingested by a new host, the cyst hatches or undergoes excystation, in the course of which the 4-nucleate amœba emerges through a pore in the cyst-wall. The subsequent (metacystic) development proceeds by successive binary fissions of the cytoplasm leading to the production of 4-uninucleate amœbulae, which grow into the typical active amœbæ.

**Cultivation and Occurrence in Lower Mammals.**—*E. nana* can be easily cultivated in the same media as *E. histolytica*. Like the entamœbæ it occurs in natural infections of monkeys.

#### v. *IODAMŒBA BÜTSCHLI* (PROWAZEK, 1912)

**Relation to Disease.**—*Iodamœba bütschli* is not pathogenic to man.

#### MORPHOLOGY AND LIFE-HISTORY

*Iodamœba bütschli* lives as a commensal in the large intestine of man and feeds on bacteria. It probably has a world-wide distribution, its incidence in most countries being relatively low (0.25–5 per cent.), though in Colombia 21 per cent. of the people examined were found to be infected.

In structure *I. bütschli* differs considerably from other species of human amœbæ. The active amœbæ (Fig. 12, e) commonly measure from 9 $\mu$  to 13 $\mu$  in diameter, the full range being 5–20 $\mu$ . Outside the human body the amœbæ are not very motile, their movements being sluggish like those of *E. coli*. The active forms are rarely encountered in the faeces; when present they soon degenerate and die at room temperature.

The nuclear structure is peculiar to this genus and varies slightly in the different stages of the amœba. In the active trophic amœba the nucleus contains a large round central karyosome surrounded

by a single layer of achromatic granules, while the nuclear membrane appears to be devoid of chromatin granules. In the living amœba the nucleus is inconspicuous.

**Encystation.**—The precystic amœbæ of *I. bütschlii* (Fig. 12, f) are similar in general appearance to the trophic amœbæ but their cytoplasm is free from food-vacuoles and their nucleus appears to be larger than that of the former, owing to the presence of several layers of achromatic granules around the karyosome.

The cysts are typically uninucleate (Fig. 12, g) but exceptionally they may contain 2 nuclei. The most characteristic feature of the cysts—which measure from  $9\mu$  to  $15\mu$ —is their irregular shape. They are generally asymmetrical and may be almost spherical or oval in outline, but frequently they are pear-shaped, bottle-shaped or kidney-shaped. The cysts have a fairly thick wall and contain a large glycogen vacuole staining dark brown and sharply outlined when treated with iodine. Chromatoid bodies are absent. The structure of the nucleus in the cysts differs from that in the free amœba. It has the appearance of a signet-ring, the large rounded karyosome being situated at one pole, while the remaining space in the nucleus is packed with the achromatic granules. In fresh unstained preparations the cysts appear as white hyaline bodies with a dull area corresponding to the glycogen vacuole, while the nucleus is invisible. Though excystation of *I. bütschlii* has been described, nothing is known regarding its metacystic development.

**Cultivation.**—*Iodamæba* is difficult to isolate in culture, but once started it will grow in media used for *Entamæba histolytica*.

**Occurrence in Lower Mammals.**—Amœbæ indistinguishable from *I. bütschlii* occur in pigs and in some monkeys but their exact relationship to the human species is not known.

#### vi. *DIENTAMÆBA FRAGILIS* JEPPE & DOBELL, 1918

**Relation to Disease.**—*Dientamæba fragilis* has been suspected of causing intestinal disorder, but evidence of its pathogenicity to man is not conclusive.

#### MORPHOLOGY AND LIFE-HISTORY

*Dientamæba fragilis* has a world-wide distribution, the incidence varying in different countries, but being especially high (over 50 per

cent. in some instances) among patients in mental hospitals. *D. fragilis* is now regarded as an aberrant flagellate but, since it is represented by amœboid forms exclusively, it is more convenient to describe it together with the true amœbæ. *D. fragilis* is one of the smallest and rarest of the human amœbæ inhabiting the large intestine. Its usual dimensions are from  $7\mu$  to  $12\mu$  in diameter, when rounded. The amœbæ are actively motile and the ectoplasm is well differentiated from the endoplasm; the food-vacuoles contain bacteria, on which *D. fragilis* feeds in the intestine. Outside the human body this amœba soon degenerates and its cytoplasm becomes highly vacuolated.

*D. fragilis* is unique among the human amœbæ in that it typically has two nuclei (Fig. 12, h, i). Though this is the normal appearance of *D. fragilis*, actually the binucleate condition represents the arrested telophase stage of mitotic division. In properly stained and fixed preparations it is seen that the nuclei have a vesicular structure, each containing 6 chromatin granules which represent the chromosomes. Furthermore, the two nuclei are connected by a fibre, representing part of the spindle (centrodesmus) left over from the division of the nucleus (Fig. 12, i). The binucleate amœba multiplies by binary fission, in the course of which the cytoplasm becomes constricted between the two nuclei, giving rise to two uninucleate amœbæ (Fig. 12, k). In these the single nucleus assumes the structure of a typical resting nucleus, the chromosomes giving rise to a large karyosome and peripheral chromatin granules lining the nuclear membrane. In the uninucleate amœba the nucleus then undergoes mitotic division which does not proceed beyond the telophase, thus giving rise to the binucleate amœba already described and representing the usual form assumed by the trophic stage of *D. fragilis*.

It should be noted that the cytological details of nuclear structure and changes just described can only be observed in stained preparations of perfectly fresh material (e.g. cultures), whereas in the fæces the appearance of the amœbæ is somewhat different. When observed in stained faecal preparations, the great majority of amœbæ seen are binucleate, with occasional uninucleate forms. In the binucleate amœbæ the chromosome granules are clumped together in what appears to be a granular karyosome, whereas the fibre connecting the two nuclei cannot usually be detected. This

appearance is due both to the rapid degeneration of the amœbæ in the feces and to the fact that some of the structural details may be obscured by bacterial inclusions.

In contrast to the other intestinal amœbæ of man, *D. fragilis* does not produce cysts and nothing is known regarding its method of transmission.

**Cultivation.**—*D. fragilis* can be readily cultivated in media used for *Entamoeba histolytica*.

**Affinities.**—In its cytology, and especially nuclear division, *D. fragilis* bears a very strong resemblance to the flagellate *Histomonas meleagridis*, which is parasitic in domestic fowls and turkeys, causing the disease known as Blackhead. *Histomonas* has both a flagellate and an amœboid stage, the latter being comparable in structure to *Dientamoeba*, which can be regarded as a closely related flagellate that has lost the power to produce flagella but has retained the amœboid stage.

### HOST-PARASITE RELATIONSHIP

Some observers have recorded various symptoms of intestinal disorder which they attributed to infection with *D. fragilis*. However, there is no evidence of invasion of the tissues or any other harmful effects produced by this amœba, which can be regarded as a harmless commensal until convincing proof of its pathogenicity is produced.

**Occurrence in Lower Mammals.**—*Dientamoeba* has been recorded in natural infections of macaque monkeys.

### DIFFERENTIAL DIAGNOSIS OF INTESTINAL AMŒBÆ

The intestinal amœbæ can be readily identified in permanent preparations of wet-fixed and stained fecal smears. However, this method is resorted to only in special cases, whereas for routine purposes it is usually sufficient to examine the parasites in fecal preparations made (a) with saline or eosin and (b) with iodine solution, as described in Chapter 16.

When active amœbæ or their cysts are present in the feces the main object of the examination is to determine whether or not the infection is due to *Entamoeba histolytica*, the only species of medical interest. The differential diagnosis of this species is based on an

TABLE 4. DIFFERENTIAL DIAGNOSIS OF INTESTINAL AMOEBAE

STAGES	CHARACTERS	<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>	<i>Endolimax nana</i>	<i>Iodamoeba butschlii</i>	<i>Dientamoeba fragilis</i>
ACTIVE AMOEBAE	Size (diameter)	10-40µ (a) Commensal form: 10-20µ (b) Tissue form: 20-40µ	20-30µ	6-12µ	9-13µ	7-12µ
	Motility	Active	Sluggish	Sluggish	Sluggish	Active
	Ectoplasm	Distinct	Indistinct	Indistinct	Indistinct	Distinct
	Food-inclusions	(a) Bacteria in commensal form (b) Red blood corpuscles in tissue forms	Bacteria	Bacteria	Bacteria	Bacteria
	Nucleus	Unstained Stained	Visible Small eccentric karyosome, coarse and irregular peripheral chromatin	Faintly visible	Faintly visible	Invisible
CYSTS	Size (diameter)	Usually less than 15µ small race 7-9µ, large race 12-15µ	Usually more than 15µ 15-20µ	6-9µ x 5-7µ	9-15µ	(Cysts absent)
	Shape	Usually spherical	Usually spherical	Ovoid or spherical	Irregular	
	Unstained (saline or eosin solution)	Invisible If present, visible hyaline rods with rounded ends	Visible Usually absent	Invisible Absent	Invisible Absent	
	Glycogen	Invisible	Invisible	Invisible	Faintly visible	
	Stained (iodine solution)	Clearly visible, number 1 to 4 (mature cysts), karyosome central	Clearly visible, number 1 to 8 (mature cysts) (rare), karyosome eccentric	Invisible or faintly visible 1 number; 1 to 4 (mature)	Faintly visible, single nucleus	
CYSTS	Chromatoids	Invisible	Invisible	Absent	Absent	
	Glycogen	May be present in cysts of all stages (1, 2, 4-nucleate), small, diffuse mass, staining brown	Usually present only in immature cysts; large well-defined mass (especially in 2-nucleate stage) staining brown	May be present in 1- and 2-nucleate stages, staining brown, absent in mature (4-nucleate) cysts	Large well-defined mass, staining dark brown	
	Nuclei	Number 1 to 4 (mature) Structure as in active amoeba but peripheral chromatin frequently forming crescentic thickening at one side	Number 1 to 8 (mature) Structure as in active amoeba	Number 1 to 4 (mature) Large eccentric round karyosome	Single nucleus, like signet-ring, large rounded karyosome at one side of nucleus, clear membrane, remaining space filled with achromatic granules	
	Chromatoids	Black or copper-coloured rods with rounded ends, or irregular bodies	Rarely seen: when present, black or copper-coloured spindles or filaments	Absent	Absent	
	Glycogen	Dissolved, leaving vacuole	Dissolved, leaving vacuole	Dissolved, leaving vacuole	Dissolved, leaving vacuole	



then gradually focussing the nuclei and counting them as they appear in view. The immature cysts of the entamæbæ may be more difficult to distinguish, but those of *E. histolytica* are usually smaller than the cysts of *E. coli*, while the binucleate cyst of the latter species frequently contains a large glycogen vacuole, displacing the nuclei to the periphery of the cyst (Fig. 10, e). As regards the 4-nucleate cyst of *E. coli*, in practice it does not give rise to confusion with the mature cyst of *E. histolytica*, since it is extremely rare. In case of doubt, further search should be made for the characteristic mature cysts. Glycogen, when present in cysts, is revealed by its brown colour. In *E. histolytica* the glycogen inclusion may be present in cysts of all stages: it appears as a small diffuse mass. In *E. coli* glycogen occurs only in immature cysts, and is especially abundant in the binucleate stage (see above); it is typically absent in the mature 8-nucleate cyst. Cysts of *Iodamæba bütschlii* are distinguished by their irregular shape and especially by the large well-defined mass of glycogen which stains dark brown. Small cysts may belong to the small race of *Entamæba histolytica* (Fig. 4, f) or to *Endolimax nana* (Fig. 12, b-d). It is sometimes possible to identify the former by the characteristic nuclear structure, but in case of doubt, permanent stained preparations should be made.

(d) Permanent Preparations.—For routine purposes it is not usually necessary to make permanent preparations, but in doubtful cases the diagnosis can be confirmed in wet-fixed stained preparations (see Chapter 18).

Plate I illustrates the appearance of mature cysts of *Entamæba histolytica* and *E. coli* as seen in faecal preparations made by the standard methods and examined in the succession outlined above.

In most cases the identification of the intestinal amæbæ presents no special difficulties, especially if cysts are available. It should be noted, however, that a correct diagnosis is usually based on a critical examination of a fair number of individuals, seen in a single or several faecal specimens, the results being arrived at by cumulative evidence.

There are certain elements in the faeces and in preparations which may give rise to confusion in the course of examination. These are dealt with in Chapter 16.

PLATE I.

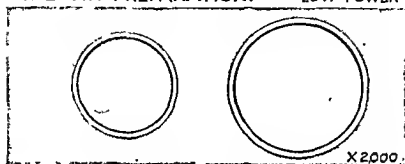
*E. histolytica*

*E. coli*



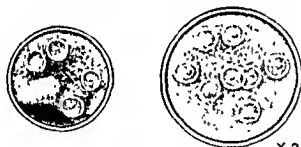
1. EOSIN PREPARATION:

LOW POWER



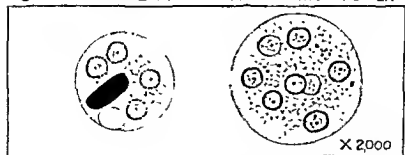
2. EOSIN PREPARATION:

HIGH POWER



3. IODINE PREPARATION:

HIGH POWER



4. STAINED PREPARATION: OIL IMMERSION

MATURE CYSTS OF ENTAMOEBAE IN FÆCAL PREPARATIONS.

(Adapted from Dobell & O'Connor, 1921, and Original)

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## CHAPTER 5

### THE FLAGELLATES

THE flagellates belong to the class MASTIGOPHORA, the main characteristic of which is the possession of locomotory organs in the form of flagella.

The body of flagellates typically has a definite form, maintained by a firm pellicle on its outer surface. Though as a rule constant, the shape of the body is sometimes subject to temporary changes due to so-called "metabolic" movements. The cytoplasm is not differentiated into ecto- and endoplasm. Some flagellates have a mouth opening (cytostome) through which formed food is ingested (e.g. *Trichomonas*, *Chilomastix*: Fig. 13, h, a), while others are devoid of a mouth, their nutrition being saprozoic, through the surface of the body (e.g. *Leishmania*, *Trypanosoma*, *Giardia*: Pl. II; Fig. 13, k). The flagella represent elastic filamentar threads which arise in the cytoplasm. Each flagellum is attached at its starting point to a small granule, known as the basal granule or blepharoplast (Fig. 19, c). The flagellum itself consists of an axial fibril, or axoneme, enclosed in a protoplasmic sheath which is continuous with the cytoplasm. The number of flagella varies in different species from one to several. Locomotion is effected by the lashing movements of the flagellum or flagella, if more than one are present, as the result of which the animal progresses in the direction of the free end of the flagellum, the corresponding end of the body being accordingly regarded as the anterior end. Some flagellates are provided with a frill-like membrane forming an extension of the pellicle on one side of the body and bearing a flagellum attached to its outer margin (Fig. 19, f). The movements of the marginal flagellum are translated to the membrane, which functions as a supplementary locomotory organ and is known as the undulating membrane.

Flagellates may possess various other internal structures, such as skeletal or supporting elements (e.g. axostyle in *Trichomonas*: Fig. 13, h-j), structures connected with locomotion (e.g. kinetoplast in trypanosomes: Fig. 19, b) and with other functions.

The flagellates multiply almost exclusively by longitudinal binary fission (Fig. 20). Many of them are known to produce cysts.

The Mastigophora are most abundantly represented by free-living forms, inhabiting both salt and fresh waters, but many are

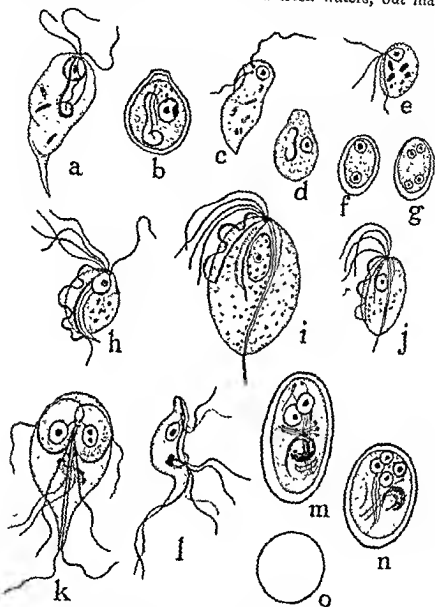


FIG. 13.—FLAGELLATES OF THE ALIMENTARY AND GENITAL TRACTS ( $\times 2,000$ ). (Adapted from Dobell and O'Connor, 1921; Wenyon, 1922; and Wenrich, 1944.)

a, b. *Chlamastix mesnili*: a. Active flagellate; b. Cyst. c, d. *Entadomonas intestinalis*: c. Active; d. Cyst. e-g. *Entadomonas hominis*: e. Active; f, g. Cysts. h. *Trichomonas hominis*; i. *T. vaginalis*; j. *T. tenax*. k-n. *Giardia intestinalis*: k, l. Active flagellates, front and side views; m, n. Cysts. o. Erythrocyte drawn to scale.

parasitic in various plants and animals, including man. Among the parasitic flagellates of man (see Table 2 and Fig. 13) six inhabit the alimentary tract and one lives in the vagina. The majority of these

appear to be harmless commensals, but two species (*Giardia intestinalis* and *Trichomonas vaginalis*) are suspected of being pathogenic. In addition to the flagellates of the alimentary and genital tracts, which are described in this chapter, human beings may be infected with flagellates living in the blood and cells of the reticulo-endothelial system and commonly known as the Hæmo-flagellates (genera *Leishmania* and *Trypanasoma*). These are dealt with in Chapters 9-11.

vii. *EMBADOMONAS INTESTINALIS* (WENYON & O'CONNOR, 1917)

Relation to Disease.—*Embadomonas intestinalis* is not pathogenic to man.

MORPHOLOGY AND LIFE-HISTORY

*E. intestinalis* (Fig. 13, c) is one of the smallest intestinal flagellates; it has an ovoid body, measuring 5-6 $\mu$  in length and 3-4 $\mu$  in breadth. The nucleus, which contains a small central karyosome, lies in the anterior end of the body, near the mouth opening (cytostome). This is an elongated depression the margins of which are supported by a curved fibril. At the anterior end of the nucleus there are two basal granules (blepharoplasts) from which 2 flagella arise: one of these is directed forwards, while the other lies partly within the oral depression. The movements of *Embadomonas* are jerky. Its food consists of small bacteria which are ingested through the mouth.

Reproduction is by binary longitudinal fission.

Encystation.—*Embadomonas* produces small pear- or lemon-shaped cysts (Fig. 13, d), measuring 4.5-6 $\mu$  in length. In addition to the nucleus the cyst contains a darkly staining looped filament, which represents the remains of the marginal fibril supporting the mouth in the free flagellate. In fresh faeces the cysts appear as opalescent whitish piriform bodies.

Habitat.—The exact part of the bowel inhabited by *Embadomonas* is not known. The incidence of infection with this flagellate in different parts of the world is very low.

Cultivation.—*Embadomonas* can be readily grown in media used for *Eutannaba histolytica*, at temperatures between 15° C. and 37° C.

viii. *CHILOMASTIX MESNILI* (WENYON, 1910)

Relation to Disease.—*Chilomastix mesnili* is not pathogenic to man.

## MORPHOLOGY AND LIFE-HISTORY

*C. mesnili* (Fig. 13, a) is one of the largest intestinal flagellates living in man. Its body is pear-shaped with the posterior end drawn out into a pointed "tail." It may measure from  $6\mu$  to  $20\mu$  in length, but is commonly  $10\text{--}15\mu$  long. This flagellate is provided with a large mouth (cytostome) in the form of a slightly spiral groove, which extends from the anterior end to about one-third or half the body length. The lateral margins of the mouth are supported by two fibrils, the right one being more pronounced and having the shape of an incomplete loop at the bottom of which is the actual oral aperture. *Chilomastix* progresses by jerky spiral movements. Food, consisting of small bacteria, is ingested through the mouth.

The nucleus is situated at the extreme anterior end of the body. It contains an achromatic network studded with chromatin granules and an eccentric karyosome. Near the anterior pole of the nucleus there is a group of basal granules (blepharoplasts) from which the flagella arise. Three of these are directed forwards, while the fourth flagellum lies in the oral groove. The anterior flagella serve for locomotion, while the oral one drives the food into the mouth.

Reproduction is by longitudinal binary fission.

Encystation.—*Chilomastix* produces cysts (Fig. 13, b) which are more or less lemon-shaped, resembling those of *Embadomonas* on a larger scale. They measure about  $7.5\text{--}8.5\mu$  in length. Within the cyst there is a single nucleus which is situated at the anterior end or near the centre of the body. The chromatin is clumped at one pole, giving the nucleus the appearance of a signet ring. Within the cytoplasm is seen a characteristically coiled darkly staining filament representing the fibril present on the right side of the mouth in the free flagellate. Other structures are not clearly visible, but occasionally a glycogen inclusion is revealed in iodine preparations. When examined fresh the cysts of *Chilomastix* appear as homogeneous bodies which do not exhibit any internal structure. They are very resistant and will remain alive for weeks outside the human body.

Habitat.—*Chilomastix* inhabits the large intestine and possibly

also the small intestine. While in diarrhœic stools both the flagellates and their cysts are passed, only the latter occur in formed stools. The incidence of infection with this flagellate varies in different localities from 1.4 to 10 per cent. Its distribution is world-wide.

**Cultivation.**—*Chilomastix* can be grown at 37° C. in media used for *Entamoeba histolytica*.

#### ix. *ENTEROMONAS HOMINIS* DA FONSECA, 1915

**Synonym:** *Tricercomonas intestinalis* Wenyon & O'Connor, 1917.

**Relation to Disease.**—*Enteromonas hominis* is not pathogenic to man.

#### MORPHOLOGY AND LIFE-HISTORY

*E. hominis* (Fig. 13, e) is a small flagellate, with an oval or rounded body measuring 4–10 $\mu$  in length and 3–6 $\mu$  in breadth. It has a somewhat piriform nucleus, with a large central karyosome, situated in the anterior end of the body. At the anterior pole of the nucleus there is a group of blepharoplasts from which 4 flagella arise. Three of these are free and directed forwards, while the fourth is directed backwards and appears to be attached to the surface of the body for some part of its length, its terminal portion projecting beyond the posterior end of the body as a free trailing flagellum. This flagellate does not possess a mouth (cytostome), food in the form of bacteria probably being taken in through the surface of the body, as in the case of amœbæ.

**Reproduction** is by binary longitudinal fission, which is sometimes arrested, giving rise to double or multiple forms.

**Encystation.**—*Enteromonas* produces elongate oval cysts (Fig. 13, f, g), measuring 6–8 $\mu$  in length and 4–5 $\mu$  in breadth. The cyst at first contains a single nucleus, which divides twice, giving rise to 4 nuclei arranged in pairs at opposite poles. The cyst may contain small chromatoid bodies.

**Habitat.**—The exact part of the intestine inhabited by this flagellate has not been established. The incidence of infection with *Enteromonas* is very low. It is probably distributed throughout the world.



Cultivation.—*Enteromonas* can be isolated in media used for *Entamoeba histolytica* but it can rarely be grown beyond the primary culture, in which it may persist for several days.

### GENUS *TRICHOMONAS* DONNÉ, 1837

Flagellates of this genus are represented in man by three species: *Trichomonas hominis*, inhabiting the intestine; *T. vaginalis* in the vagina, and *T. tenax* in the mouth.

The trichomonads (Fig. 13, h-j) have a more or less ovoid body, with a single oval nucleus, from 3 to 5 free anterior flagella and one flagellum which runs backwards along the margin of an undulating membrane. These flagellates possess a stiff supporting rod, or axostyle, passing through the middle of the body and protruding from its posterior end in the form of a tail-like process. At the anterior end of the body there is a more or less developed mouth or cytostome, through which solid food is ingested. Other structural details are dealt with in the description of the separate species.

The trichomonads reproduce by longitudinal binary fission. They do not form cysts. Trichomonads are widely distributed in various lower animals, as well as in man. They occur in fishes, amphibia, reptiles, birds and lower mammals. Some, like *T. columba* of pigeons and *T. fetus* of cattle, are pathogenic to their hosts.

#### x. *TRICHOMONAS HOMINIS* (DAVAINE, 1860)

Relation to Disease.—*Trichomonas hominis* is not pathogenic to man.

#### MORPHOLOGY AND LIFE-HISTORY

*T. hominis* (Fig. 13, h) is one of the commonest intestinal flagellates of man. Its body is somewhat oval or pear-shaped but is subject to metabolic changes of form. It usually measures about  $8\mu$  in length, with a range from  $5\mu$  to  $14\mu$ . The oval nucleus, which lies in the anterior half of the body, has a small karyosome and chromatin granules lining the inner surface of the nuclear membrane. At the anterior end of the body there is a group of basal granules (blepharoplasts) giving rise to a variable number of flagella. The

majority of flagellates possess 5 free flagella directed forwards, but some have only 3 or 4 anterior flagella. One side of the body is occupied by an undulating membrane which in this species extends to the posterior end of the body and runs a somewhat spiral course. The undulating membrane carries a flagellum which starts from the anterior group of blepharoplasts, runs in a wavy line along the external margin of the membrane and ends posteriorly as a free trailing flagellum extending beyond the hind end of the body. The base of the undulating membrane is supported by a filament, the basal fibre or costa. Through the centre of the body, in its longitudinal axis, passes a stout faintly stainable rod, the axostyle, which arises anteriorly from the group of blepharoplasts, bends round one side of the nucleus, runs to the posterior end of the body and protrudes outside the body as a pointed caudal process. The axostyle appears to be skeletal in function.

At the side of the axostyle, at its anterior end, there is a slit-like mouth or cytostome, through which food, consisting of bacteria, starch and sometimes erythrocytes, is ingested.

When examined alive, *T. hominis* is seen as a colourless body, usually rotating around its long axis, with the anterior flagella lashing rapidly, while the undulating membrane exhibits a rippling motion with waves continuously passing from its anterior end backwards. The axostyle can be seen as a spike protruding from the hind end of the body.

Reproduction is by binary longitudinal fission.

Transmission.—*T. hominis* is capable of surviving at room temperature for at least a fortnight. It does not encyst but may become rounded and lose its flagella, the rounded flagellate being a highly resistant form equivalent to a cyst. Transmission is effected by a new host swallowing either the active flagellates or the rounded forms, both of which pass unharmed through the mouth and stomach into the bowel.

Habitat.—*T. hominis* lives in the large bowel. It is frequently passed in diarrhoeic stools and, both on this account and because it occasionally ingests red blood corpuscles, it has been suspected of being pathogenic to man. However, there is no evidence of its being anything more than a harmless commensal. The distribution of this flagellate is world-wide, its incidence being up to 6 per cent. in temperate regions and considerably higher in the tropics. A

flagellate indistinguishable from this species and infective to man occurs in macaque monkeys.

Cultivation.—*T. hominis* grows readily at 37° C. in media used for the cultivation of *Entamoeba histolytica*; it can survive for a fortnight in cultures kept at room temperature and longer in cold storage.

#### xi. *TRICHOMONAS TENAX* (MÜLLER, 1773)

Synonyms : *T. elongata* Steinberg, 1862; *T. buccalis* auctorum.

Relation to Disease.—*Trichomonas tenax* is not pathogenic to man.

#### MORPHOLOGY AND LIFE-HISTORY

*T. tenax* (Fig. 13, j) is somewhat similar in structure to the intestinal trichomonad. It has an oval or piriform body measuring 5–12 $\mu$  in length. The oval nucleus, lying near the anterior end of the body, contains several scattered chromatin granules and a small karyosome. Arising from a group of blepharoplasts at the foremost end of the body, there are 4 free flagella directed forwards and one flagellum running backwards along the margin of an undulating membrane, which extends almost to the posterior end of the body. The marginal flagellum has no free portion but terminates at the posterior end of the membrane. As in the case of *T. hominis*, the base of the undulating membrane is supported by a basal fibre (costa). The axostyle is more slender than that of *T. hominis* and projects from the posterior end of the body as a pointed rod. At the anterior end of the body, adjoining the axostyle, there is a narrow mouth (cytostome), through which solid food, chiefly in the form of leucocytes, is ingested. Between the axostyle and the undulating membrane there is a faintly staining parabasal body, starting in the region of the blepharoplasts and extending to the middle of the body. The function of the parabasal is unknown. The oral trichomonad does not produce cysts, its transmission being probably effected by direct contact or by contamination with infected saliva as in the case of the oral amœba, *Entamoeba gingivalis*.

Habitat.—*T. tenax* lives in the mouth, and is more commonly found in persons with oral disease or dental disorder than in individuals with a well-kept mouth. It has been found throughout the

world, in cases of dental caries, pyorrhœa, in infections of the gums, throat and lungs, and has been recovered from sputum. The incidence of infection may be up to 26 per cent. in persons with diseased mouths and up to 11 per cent. in those with healthy mouths. Though *T. tenax* is frequently associated with a diseased condition of the mouth, there are no grounds for believing it to be the cause of any disorder.

Cultivation.—*T. tenax* has been cultivated in media used for intestinal amœbæ.

## xii. *TRICHOMONAS VAGINALIS* DONNÉ, 1837

Relation to Disease.—*Trichomonas vaginalis* is thought by some authorities to be responsible for inflammatory conditions of the vagina ("trichomonas vaginitis") but there is no conclusive evidence of its pathogenicity.

### MORPHOLOGY AND LIFE-HISTORY

*T. vaginalis* (Fig. 13, i) is the largest of the human trichomonads, its average length being about  $13\mu$ , with a range from  $7\mu$  to  $23\mu$ . Its body is typically oval, but it tends to be rounded. The oval nucleus, which is in the anterior part of the body, contains scattered chromatin granules and a small central karyosome. As in the two other human trichomonads, there is a group of blepharoplasts at the anterior end of the body which give rise to 5 flagella: four of these are free flagella directed forwards, while the fifth runs along the margin of the undulating membrane. This membrane is shorter than in the other trichomonads and usually does not extend beyond one-third or two-thirds of the body-length. At the base it is supported by a fibre (costa). The marginal flagellum has no free portion but terminates at the posterior end of the undulating membrane. The axostyle of *T. vaginalis* is more slender than in the other trichomonads: after starting from the blepharoplasts it bends along the side of the nucleus, and passes through the middle of the body, projecting beyond its posterior end as a pointed rod. The mouth (cystostome) has the form of a very narrow slit adjoining the anterior end of the axostyle and is usually inconspicuous. There is a well-developed parabasal body lying close to the nucleus beneath the undulating membrane. This trichomonad feeds on

leucocytes and probably also on glycogen present in the vaginal epithelium. In fresh preparations of the vaginal exudate *T. vaginalis* is recognized by the characteristic rippling motion of the undulating membrane and the lashing anterior flagella.

Like the other trichomonads, *T. vaginalis* does not produce cysts.

Cultivation.—Various special media have been devised for the cultivation of *T. vaginalis*, both with and without bacteria, but generally the results are unsatisfactory, since very little is known about its growth requirements.

### HOST-PARASITE RELATIONSHIP

*T. vaginalis*, which has a world-wide distribution, lives in the vagina of women, where it is found in the vaginal exudate. The incidence of infection with this trichomonad may be from 10 to 40 per cent. of unselected cases examined, but in women suffering from discharge due to leucorrhœa and various forms of vaginitis, as well as in cases of gonorrhœa, it may be present in up to 70 per cent. of cases. The incidence is therefore particularly high among women attending gynaecological and venereal clinics.

*T. vaginalis* requires for its development an acid medium with an average pH of 5.5; it is accordingly rarely found in adult women with a normal acidity of the vaginal contents at pH 4-4.5, nor does it occur in immature girls and old women, whose vaginal discharge approaches neutrality. The normal reaction within the vagina is maintained by the production of lactic acid by Döderlein's *Lactobacillus* at the expense of the glycogen which is commonly present in the epithelial cells of the vagina during puberty, but is scanty in childhood and tends to disappear with the onset of the menopause. Trichomonad infections are usually associated with a decrease in the number or disappearance of Döderlein's bacilli. These are replaced by various other microorganisms, some of which are presumably capable of reducing the acidity of the vaginal contents, producing conditions favourable to the growth of the flagellates.

Pathogenesis.—Most gynaecologists regard *T. vaginalis* as a pathogenic organism, responsible for various conditions associated with its presence in the vagina and described under the name "trichomonas vaginitis." However, there is no absolute proof of the causal relationship between this trichomonad and vaginal disorder, since this might be due to bacteria or spirochaetes which

are commonly associated with a diseased condition of the vagina, while the flagellates merely multiply more intensely in a favourable environment. In favour of this view is also the fact that symptomless carriers of *T. vaginalis* are more common than cases of vaginitis. On the other hand, it has been demonstrated, by experimental inoculation of uninfected women, that this flagellate sometimes produces an inflammatory reaction in the vaginal mucosa. Though the question regarding the pathogenicity of *T. vaginalis* has not yet been solved, it should be borne in mind that a closely related flagellate, *T. fetus*, which is parasitic in cattle, is definitely pathogenic. It is the cause of a venereal disease of bovines, transmitted from infected bulls to cows, in which it lives in the vagina and invades the uterus, causing abortions, delayed conception and other symptoms.

**Transmission.**—The method of transmission of *T. vaginalis* is not yet known. However, there have been reports of infection of the urethra in males, and it is conceivable that the trichomonad might be transmitted from male to female during the sexual act, as in the case of bovine trichomoniasis.

### DIAGNOSIS

*Trichomonas vaginalis* occurs in the vaginal discharge of females, and may occasionally be found beneath the prepuce or in the anterior part of the urethra in males.

The flagellates can be detected in fresh or stained preparations of the vaginal discharge, a sample of which can be obtained on the gloved finger in the course of examining the patient, or by dipping a swab into the vaginal contents.

For FRESH PREPARATIONS a drop of the discharge is mixed with a small amount of serum diluted with normal saline (1 : 10) or with Ringer's solution. A drop of the fluid is placed on a slide and a coverslip is applied, after which the preparation is examined under the microscope. The trichomonads are seen as clear refractile bodies and can be recognized by the characteristic movements of the flagella and the undulating membrane.

STAINED PREPARATIONS can be made by both the "dry" and the "wet" methods of fixation (see Chapter 18). A very crude method, though satisfactory for rapid diagnosis, is to make a smear on a slide with material adhering to the gloved finger after examina-

tion of the patient. The smear is dried in the air and stained with one of the Romanovsky stains (see Chapter 17). Although in such preparations the flagellates are usually distorted and badly stained, they reveal sufficient structural features for the identification of the parasites by an experienced worker. Much better results are obtained by mixing the vaginal discharge with a little dilute serum and making a very thin film on a warmed (but not too hot) slide. The smear is then treated like a thin blood film (see Chapter 17). A careful search of the stained film usually reveals some flagellates in which all the morphological features are clearly visible. However, for finer cytological details smears should be fixed by the "wet" method and stained with hæmatoxylin (see Chapter 18).

### xiii. *GIARDIA INTESTINALIS* (LAMBL, 1859)

Synonym : *G. lamblia* Stiles, 1915.

Relation to Disease.—*Giardia intestinalis* is regarded by many clinicians as a pathogenic parasite causing a variety of disturbances in the intestine and gall-bladder, known collectively as GIARDIASIS. However, there is no conclusive evidence of its pathogenicity.

### MORPHOLOGY AND LIFE-HISTORY

*G. intestinalis* (Fig. 13, k, l) has a characteristic appearance which differs markedly from that of all the other intestinal flagellates of man. Its body has the shape of a pear from the thicker end of which a portion has been cut off obliquely, leaving a flattened side which represents the ventral surface of the body, while the opposite convex side forms the dorsal surface. When viewed from the front, the flagellate looks like a tennis racket without the handle. *Giardia* is bilaterally symmetrical, having a double set of structures, one on the right side of the median line, the other on the left. The anterior end of the body is broad and rounded, while posteriorly the body gradually tapers to a tail-like point. On the ventral surface of the body there is a reniform depression, supported by two curved fibres. This depression, by means of which the flagellate attaches itself to the mucosa, is known as the "sucking disc" or "sucker." The body of the flagellate, without the flagella, measures 10–18 $\mu$  in length.

The internal structure of *Giardia* is very complicated: there are 2 nuclei and 4 pairs of flagella, as well as some other elements, but no mouth. The nuclei lie in the anterior end of the body, beneath the "sucker" on either side of the median line. They are oval in shape and each contains a central karyosome. Between the two nuclei there is a group of 6 basal granules (blepharoplasts) from which 3 pairs of flagella arise. The first pair of flagella start from the foremost couple of blepharoplasts, cross each other anteriorly, pass to the opposite sides of the "sucker" and emerge from the body as free flagella. The second pair of flagella start from another pair of blepharoplasts, situated between the nuclei, pass down to the posterior edge of the "sucker," where they diverge to the sides of the body and emerge as free lateral flagella. Another pair of blepharoplasts, situated in the median line between the foremost pair of blepharoplasts, give rise to two filaments running in a straight line to the posterior end of the body from which 2 free flagella (the third pair) emerge. There is some controversy about the nature of these intracytoplasmic filaments, which some authors regard as a pair of fine supporting fibres or axostyles, while others interpret them as two flagella each ending in a free flagellum posteriorly. The fourth pair of flagella start from a couple of blepharoplasts situated in the depression of the posterior margin of the "sucker" and emerge from the ventral surface of the body. In the middle of the body, dorsally to the "axostyles," there is a single or double somewhat comma-shaped deeply stainable body known as the parabasal, the functions of which are unknown.

The appearance of *Giardia* in a fresh preparation is unmistakable. When swimming the flattened piriform flagellate sways from side to side with the four pairs of flagella lashing around.

Reproduction is by longitudinal binary fission. Food, chiefly in the form of carbohydrates in solution, is absorbed through the surface of the body.

Encystation.—*Giardia* produces characteristic cysts (Fig. 13, m, n) which are oval or elliptic in shape, measuring 10–14 $\mu$  in length, and have a thick wall. The encysted flagellate is also oval but does not fill the entire cyst, one or both ends of the body being usually detached from the cyst-wall. Within the cyst the flagella, axostyles and supporting fibres of the "sucker" break away and gradually disintegrate, their remains being visible as streaks and filaments



scattered in the cytoplasm; the most prominent structures seen in the cyst are the comma-shaped parabasal body and the nuclei. The two nuclei are situated at one pole of the cyst where they soon divide, giving rise to 4 nuclei. The cysts of *Giardia* can be easily recognized both in fresh and iodine-stained preparations by their oval shape and inner structure. They can remain viable in moist faeces for about a fortnight. As in other intestinal protozoa which produce cysts, transmission of *Giardia* takes place through the mouth, when food or drink contaminated by cysts are swallowed by a new host. It has been reckoned that in some cases several million cysts per gramme of faeces may be passed. Excystation has not been observed.

Cultivation.—There are no reliable methods of cultivating *Giardia*.

### HOST-PARASITE RELATIONSHIP

Habitat.—*G. intestinalis* lives in the small intestine, chiefly in the duodenum, where it attaches itself by means of the "sucker" to the epithelial lining of the gut and feeds on the semi-digested food of its host. *Giardia* is discharged in the faeces almost exclusively in the form of cysts, but free flagellates may occasionally appear in diarrhoeic stools.

Incidence.—*Giardia* has a world-wide distribution, being a common parasite of man, especially in children under the age of ten, the incidence of infection varying from 5 to 16 per cent. of people examined.

Pathogenesis.—There is a widespread tendency among clinicians to regard *Giardia* as a pathogenic parasite responsible for various intestinal disturbances and disorders of the gall-bladder. It is thought that this flagellate may interfere with absorption of fats, the presence of which in the lower region of the bowels causes persistent diarrhoea with characteristic fatty stools (steatorrhoea) resembling those passed in cases of sprue. These flagellates are also believed to be able to invade the gall-bladder, causing inflammation (cholecystitis) and affecting the bile-ducts. However, there is no conclusive evidence of the aetiological rôle of *Giardia* in these conditions, for it occurs quite commonly among healthy children and adults, while similar intestinal disorders are found in persons not harbouring *Giardia*. The relation of giardial infection to an inflammatory condition of

the gall-bladder is still more doubtful, for in many cases when removed at operation the gall-bladder has failed to reveal any flagellates, though these were present in the duodenal contents of the patient. In the few cases in which flagellates have been recovered from the gall-bladder their presence there might have been due to secondary invasion. In general, the symptomatology of giardiasis—or lambliasis, as it is sometimes called—is not well defined and it is doubtful if this flagellate is responsible for all the disorders attributed to it. However, since *Giardia* attaches itself in large numbers to the mucous membrane of the duodenum and ileum, where it lives at the expense of the semi-digested food of its host, it cannot be regarded as an entirely harmless commensal, like the other intestinal flagellates. It is therefore conceivable that under suitable conditions it may interfere with the absorption of food and cause both mechanical irritation and functional disturbance.

**Occurrence in Lower Animals.**—Species of *Giardia* occur in vertebrate animals of all classes and, except for minor structural details, resemble the human flagellate.

### DIAGNOSIS

*Giardia* can be detected by microscopic examination of the faeces (see Chapter 16). As a rule only cysts are passed but in diarrhæic conditions the stool may contain free flagellates. These can be demonstrated in material obtained by means of a duodenal tube (duodenal drainage).

## CHAPTER 6

### THE COCCIDIA

COCCIDIA belong to the class SPOROZOA (see Chapter 2), which comprises exclusively parasitic protozoa and includes also malaria parasites, the life-cycle of which has many features in common with that of coccidia. Coccidia are intracellular parasites, which occur in various groups of invertebrate and vertebrate animals. Their entire life-cycle usually passes in the same host, in which they may inhabit different organs, the alimentary canal and accessory organs (e.g. the liver) being more commonly affected.

Among the coccidia there are a number of pathogenic forms, causing serious disease known as COCCIDIOSIS in various domestic animals, e.g. in cattle, rabbits and fowls. Man is known to harbour at least one, and possibly two, species of coccidia belonging to the genus *Isospora*. The complete life-history of the human coccidia has not been observed but, since the course and stages of development of most coccidia are similar, it can be illustrated by a description of the typical life-cycle of an *Isospora* as it proceeds in the alimentary tract (Fig. 14). The parasite usually lives within the epithelial cells of the gut. Its life-cycle consists of several asexual generations, followed by a sexual generation, and terminating in the formation of an encysted stage, or oocyst, which is discharged in the faeces and serves to infect a new host when swallowed.

The youngest stage of the coccidium is a rounded form (Fig. 14, a) with a single vesicular nucleus, lying in an epithelial cell. This form, known as a TROPHOZOITE, gradually increases in size (Fig. 14, b) and undergoes a process of multiplication by *schizogony*. In the course of schizogony the nucleus of the parasite, now known as a SCHIZONT, divides repeatedly by a rapid succession of mitotic divisions into two, with new spindles being formed before the preceding division is completed, so that an impression of multiple nuclear divisions proceeding simultaneously is produced. After the requisite number of nuclei has been produced (Fig. 14, c), a portion of cytoplasm is budded off around each daughter-nucleus and the schizont divides, or undergoes *segmentation*, into as many vermiform young forms, or MEROZOITES (Fig. 14, d), as there were

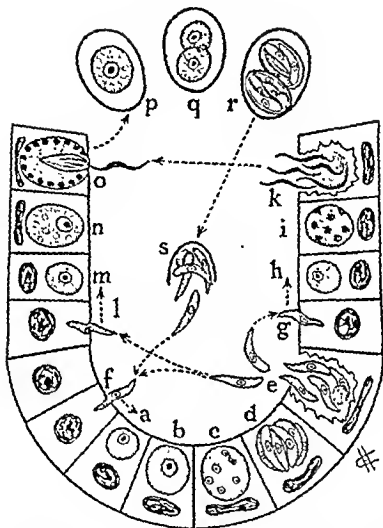


FIG. 14.—LIFE-CYCLE OF A COCCIDIUM (*Isospora*). (Original.)

Diagrammatic representation of adjacent walls of two intestinal villi. a-c, f, a. Asexual cycle: a, b. Young and fully grown trophozoites; c. Schizont with 5-6 nuclei; d. Segmented schizont with merozoites; e. Release of merozoites into the lumen; f. Merozoite entering new epithelial cell. g-s. Sexual cycle: g. Merozoite ( $\sigma$ ) entering epithelial cell; h. Young microgametocyte; i. Nuclear division in microgametocyte, leading to production of microgametes (k); l. Merozoite ( $\varphi$ ) entering epithelial cell; m-n. Growth of macrogametocyte; o. Fertilization of macrogamete; p. Oocyst with zygote; q. Oocyst with 2 sporoblasts; r. Ripe oocyst (infective stage) with 2 sporocysts (spores), each containing 4 sporozoites; s. Sporozoites escaping from ruptured sporocyst to invade new epithelial cells (f).

nuclei in the mature schizont. The merozoites, which are motile, escape from the infected cell (Fig. 14, e) into the lumen of the gut and invade new epithelial cells (Fig. 14, f), in which the development

just described and representing the asexual cycle is repeated (Fig. 14, a-c). The asexual cycle may consist of many generations multiplying by schizogony, but at a certain stage of the infection the merozoites become sexually differentiated into male and female forms, which initiate the sexual cycle of development.

After entering new host-cells (Fig. 14, l, g) the sexual forms of merozoites become rounded like the trophozoites, from which they are at first indistinguishable. The female forms are known as MACROGAMETOCYTES (Fig. 14, m) and the male ones as MICROGAMETOCYTES (Fig. 14, h). The microgametocyte first increases in size, then its nucleus divides repeatedly (as in schizogony) (Fig. 14, i), and a large number of comma-like or filamentar daughter-individuals, known as MICROGAMETES, are produced from the surface of the body, the greater part of which is left behind as a residuc, while the microgametes themselves escape into the lumen of the gut (Fig. 14, k). As regards the macrogametocyte, it increases in size, without division of the nucleus, until it almost fills the host-cell (Fig. 14, n). The cytoplasm of the macrogametocyte is filled with refractile globules which later arrange themselves on the periphery of the body, while the nucleus is drawn out to one pole of the female, which is now mature and is known as the MACROGAMETE. The microgametes, which correspond to the spermatozoa of higher animals, are attracted to the macrogametes, which correspond to the ova of metazoa. One of the male gametes penetrates into the female gamete (Fig. 14, o) and fertilizes it, the nuclei of the two uniting. After fertilization has taken place the peripheral globules present in the macrogametocyte fuse and give rise to a tough membrane, or cyst, surrounding the body, while the fertilized female contracts into a rounded body—the ZYGOTE. The encysted zygote, now known as the OOCYST (Fig. 14, p), drops into the lumen of the gut and is discharged with the feces, its further development taking place outside the host's body. In the external medium the oocyst develops by a process known as sporogony, which usually takes several days. First the zygote divides into two SPOROBlasts (Fig. 14, q), each of which becomes invested with a cyst of its own—the SPOROcyST. Within each sporocyst, or spore, there arise by further division of the body 4 vermiform SPOROZOITES (Fig. 14, r). In the course of this development part of the cytoplasm of the zygote and sporoblasts may be left behind as oocystic and sporocystic residues respectively.

With the formation of the sporozoites the oocyst attains maturity and is capable of infecting new hosts. When a fully developed oocyst is swallowed by a new host, its wall and that of the spores (sporocysts) are ruptured, releasing the 8 sporozoites into the lumen of the gut (Fig. 14, s). The sporozoites penetrate into the epithelial cells of the mucosa and start the asexual cycle of development (Fig. 14, a-e), as already described.

In the example given above the oocysts are invested with a thick hard wall and are discharged in the faeces in an immature state, their development (sporogony) being completed outside the host's body. This type of development is characteristic of coccidia living in the cells of the mucosa. However, some coccidia occur in the subepithelial tissues in which their entire development, including sporogony, is completed. In such coccidia the oocysts when voided with the faeces are already mature, containing fully developed sporocysts. The oocyst wall in such cases is usually very thin and soft, closely enveloping the sporocysts, but sometimes it ruptures before leaving the host's body, with the result that the sporocysts are found free in the faeces.

The oocysts of coccidia are the most resistant forms known among protozoa. They can remain alive and will continue to develop in acids and various strong reagents. When ingested by an animal which is not their natural host, the oocysts may pass through its intestine undamaged and remain viable when passed in its stools. Thus, the oocysts of coccidia of fish, when ingested by man, have been known to pass unaltered through the human intestine. When recovered from the stools, they have been misinterpreted as belonging to human parasites (genus *Eimeria*). Though resistant to chemicals, coccidial oocysts are destroyed when exposed to a temperature of 56° C. or to desiccation.

The various genera of coccidia are differentiated by the structure of the mature oocysts. The most important genera occurring in mammals are *Isoospora* and *Eimeria*, which differ in the following characters. The oocyst of *Eimeria* (Fig. 15, f) contains 4 sporocysts with 2 sporozoites in each, while the oocyst of *Isoospora* (Fig. 15, c, d) contains 2 sporocysts, each with 4 sporozoites.

### COCCIDIA OF MAN

The coccidia found in man belong to the genus *Isoospora*. There is still some doubt whether they are represented by one or two species.

xiv. *ISOSPORA BELLI* WENYON, 1923

Relation to Disease.—*I. belli* gives rise to COCCIDIOSIS characterized by enterocolitis.

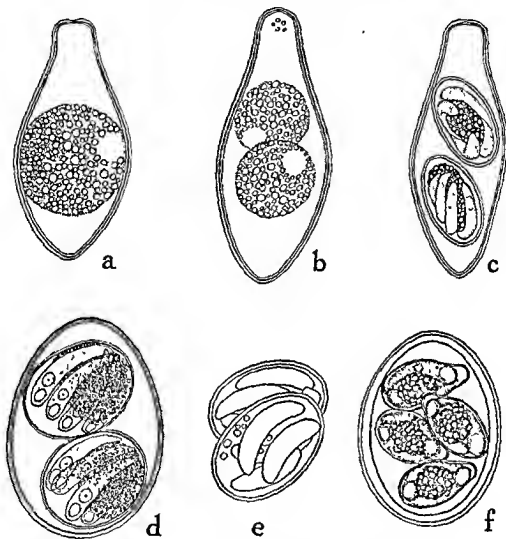


FIG. 15.—COCCIDIA ( $\times 1,800$ ). (After Dobell and O'Connor, 1921; Wenyon, 1923; Reichenow, 1929; and Hoare, 1927.)

a-c. *Isospora belli* from man: a. Oocyst with zygote; b. Oocyst with 2 sporoblasts; c. Mature oocyst with 2 sporocysts, each containing 4 sporozoites and residual body; d. *Isospora bigemina* var. *canis* from dog, mature oocyst; e. *Isospora hominis* (? = *I. bigemina* var. *canis*) from man: two mature sporocysts (oocyst wall invisible); f. *Elmeria* from ferret: mature oocyst with 4 sporocysts, each containing 2 sporozoites.

## MORPHOLOGY AND LIFE-HISTORY

The stages of development of *I. belli* in the human intestine are unknown, the only forms observed up to the present being the

oocysts which are passed in the stools. The oocyst is thick-walled; it has an elongated somewhat asymmetrical shape, narrowed at one end in the form of a neck and rounded at the poles. Its dimensions are  $25-33\mu$  in length and  $12.5-16.5\mu$  in breadth. The oocysts leave the host's body in the zygote stage (Fig. 15, a) and develop in the external medium, completing their sporogony in from 1 to 4 days, in the course of which first 2 sporoblasts (Fig. 15, b) and finally 2 oval sporocysts, or spores, are formed, each measuring  $12-14\mu$  in length and  $7-9\mu$  in breadth. Each mature spore contains 4 sporozoites and a residual mass of cytoplasm (Fig. 15, c). Though nothing is known regarding the development of *I. belli* in the intestine, it is probable, on analogy with other species of this genus in which sporogony takes place outside the body of the host, that it occurs in the epithelial cells of the villi of the small intestine, as outlined above (Fig. 14). Experimental infections of volunteers have shown that the life-cycle takes from 9 to 16 days.

### HOST-PARASITE RELATIONSHIP

As in the case of other coccidia, infection with *I. belli* takes place when food or drink contaminated with the oocysts is swallowed. The incidence of infection with *I. belli* is relatively low, only several hundred cases having hitherto been recorded from different parts of the world, all of them in countries with a warm, subtropical or tropical climate.

**Pathogenesis.**—Though the clinical course of human coccidiosis has not been fully studied, observations on natural and experimental infections with *I. belli* indicate that the disease is self-limited, lasting not more than 3 weeks, with symptoms of acute enterocolitis. Since *Isoospora* is a tissue parasite it presumably causes a certain amount of damage to the intestinal wall.

### DIAGNOSIS

Diagnosis of coccidiosis is based on the finding in the stools of the characteristic oocysts of *I. belli*, the complete development of which can be observed *in vitro*, employing the method described in Chapter 19.



xv. *ISOSPORA HOMINIS* (RIVOLTA, 1878)

While the status of *I. belli* as a human parasite is well established, the question regarding the occurrence in man of a second species of *Isospora* is still unsettled.

The facts are as follows. The earliest records, going back to the last century, refer to the finding in the subepithelial tissues of the human intestine of a coccidium which was similar to the final stages of development of the canine coccidium, *Isospora bigemina* var. *canis* (Fig. 15, d), represented by oocysts containing two bodies (? spores) and found in the same situation. This human coccidium was subsequently named *I. hominis*. What appears to be the same coccidium was rediscovered some twenty years ago, when two sporocysts held together by an invisible oocystic membrane were seen in human faeces (Fig. 15, e). The measurements of these sporocysts ( $16 \times 10.5\mu$ ) correspond closely to those of the canine *Isospora* ( $15 \times 10\mu$ ), while their mature state suggests that—as in the earliest recorded cases—they had probably completed their development in the subepithelial tissues of the gut. Thus, the only data regarding *I. hominis* are that it occurs in the subepithelial tissues of the gut, where its development is completed with the production of a thin-walled oocyst containing two sporocysts, which have the same dimensions as the *Isospora* of the dog.

Some authors believe that *I. hominis* and *I. belli* actually represent the same species and that the former is the correct name for the human coccidium. However, there is not sufficient evidence to support the identity of these two species. Moreover, their geographical distribution is different, since *I. hominis* has only been reported from countries with a temperate climate, whereas all the cases of infection with *I. belli* occurred in warm countries. On the other hand, it is conceivable that *I. hominis* is actually a coccidium of the dog, the oocysts of which might accidentally be swallowed by man and either start an infection or pass unaltered in the faeces.

## CHAPTER 7

### THE CILIATES

THE last group of human intestinal protozoa to be considered belong to the class CILIOPHORA (see Chapter 2), which comprises the most highly organized forms and is abundantly represented in nature by both free-living and parasitic species (Figs. 16 ; 18, f, g, h).

The body of a ciliate has a constant shape, the cytoplasm being differentiated into a thin layer of ectoplasm and the main mass of endoplasm. The body is typically covered with a coat of hair-like cilia, each of which arises from a basal granule. The cilia are arranged in longitudinal rows which give the surface of the body a striated appearance. The cilia beat in unison and serve for locomotion. The majority of ciliates are provided with a mouth (cytostome) and sometimes accessory oral structures. In the endoplasm there are two kinds of nuclei: a large MACRONUCLEUS which is of the massive type (Fig. 1, C) and is vegetative in function, and a small MICRONUCLEUS of the vesicular type (Fig. 1, B) which is a generative nucleus. There are also one or more contractile vacuoles in the cytoplasm. The vacuoles, representing excretory organs, periodically become filled with fluid which is discharged through a pore on the surface of the body.

Food-particles are ingested through the mouth; when they enter the endoplasm they are surrounded by a drop of fluid to form food-vacuoles in which the food is digested. The food-vacuoles circulate in the body until finally their undigested contents are voided through a permanent anal opening (cytopyge).

Multiplication in ciliates takes place by transverse fission of the body, preceded by binary division of the nuclei, the micronucleus dividing mitotically, while the macronucleus divides amitotically by simple constriction into two halves.

Most ciliates are capable of encysting. Within the cyst they become rounded and lose their cilia and some of the inner structures.

Ciliates are sexually hermaphrodites and undergo a peculiar form of sexual union (syngamy) known as CONJUGATION. In the course of conjugation two individuals become attached to each other, after which an exchange of nuclear elements takes place. In each

of the partners, or conjugants, the micronucleus divides twice, giving rise to 4 micronuclei, while the macronucleus disintegrates. Then 3 of the micronuclei degenerate, leaving 1 micronucleus in each conjugant. This micronucleus divides again, after which 1 micronucleus in each conjugant passes into the body of the other one where it fuses with the micronucleus which had remained stationary, the process being equivalent to fertilization. The two conjugants then separate and become independent. In these ciliates the normal nuclear constitution is restored by a nuclear division giving rise to a micronucleus and a macronucleus.

The only ciliate parasitic in man belongs to the genus *Balantidium*.

#### xvi. *BALANTIDIUM COLI* (MALMSTEN, 1857)

Relation to Disease.—*Balantidium coli* is pathogenic to man, causing a disease known as BALANTIDIOSIS, with ulceration of the intestinal wall and symptoms of colitis, diarrhoea or dysentery (balantidial dysentery).

#### MORPHOLOGY AND LIFE-HISTORY

*B. coli* (Fig. 16) is the only authentic ciliate parasitic in man; it is also the largest of the human intestinal protozoa. The body of the ciliate (Fig. 16, a) is oval in shape, measuring 60–70 $\mu$  in length and 40–60 $\mu$  in breadth, but smaller and larger individuals are also encountered. At the anterior end there is a groove (peristome) leading to the mouth opening (cytostome), from which a funnel-shaped gullet, or cytopharynx, runs inwards, ending in the anterior third of the body. The body is evenly covered with short cilia which are arranged in longitudinal, slightly spiral rows, so that the surface of the ciliate appears to be striated. Within the oral region there is a row of longer cilia.

The kidney- or sausage-shaped macronucleus is situated in the middle of the body together with the micronucleus, which is a small spherical structure lying in a depression of the large nucleus. There are two contractile vacuoles, situated at one side of the body, one in the middle, the other near the posterior end. At the posterior extremity there is a short excretory canal leading to the anal opening (cytopyge) on the surface of the body.

Reproduction.—Like all ciliates, *Balantidium* multiplies by trans-

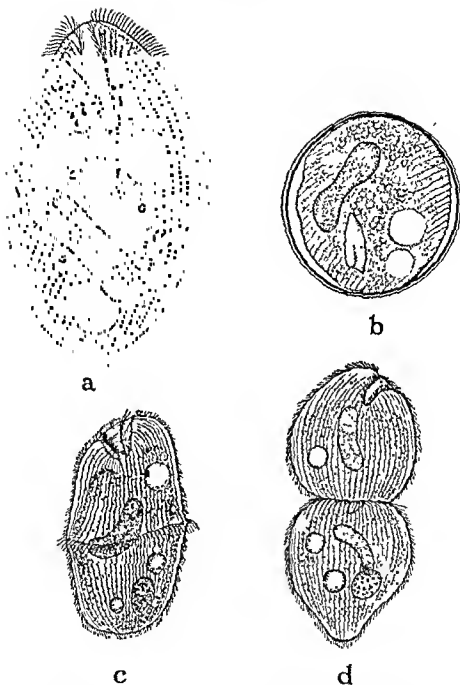


FIG. 16.—*Balantidium coli* ( $\times$  ca. 1,000). (Adapted from Wenyon, 1926; Dobell and O'Connor, 1921; and Hartmann and Schilling, 1917.)

a. Active ciliate; b. Cyst; c, d. Dividing forms.

verse binary fission (Fig. 16, c, d), in the course of which the posterior daughter-individual reconstructs the mouth and other structures, while the anterior individual re-forms the posterior end of the body. Conjugation has also been described in this ciliate.

**Nutrition.**—Food, which consists of faecal particles, starch, red blood corpuscles and fragments of the host's tissues, is ingested through the mouth. The food passes down the gullet and on reaching the endoplasm the ingested particles are enclosed in food-vacuoles. These circulate in the body until the food is digested, after which the undigested remnants are discharged through the anal aperture.

**Encystation.**—When about to encyst *Balantidium* becomes rounded, eliminates all food-vacuoles from its body, and secretes a thick cyst-wall. In the cyst the ciliate loses its ciliary covering, but a contractile vacuole may remain active for some time, while the macronucleus is the most conspicuous inclusion. The cysts (Fig. 16, b) are spherical or ovoid, measuring 50–60 $\mu$  in diameter. Occasionally they contain two individuals. The cysts are discharged in the faeces and may survive in moist stools for several weeks but are rapidly killed by desiccation. When swallowed by a human being the cyst hatches in the intestine, liberating the free ciliate, the various inner structures of which are presumably re-formed before excystation takes place.

**Cultivation.**—*B. coli* can be grown at 37° C. in some of the media used for the cultivation of *Entamoeba histolytica*.

### HOST-PARASITE RELATIONSHIP

*B. coli* occupies a peculiar position among the human intestinal protozoa, in that it is a parasite common to man and the domestic pig, the latter being regarded as the natural host, while man is only an incidental host of this ciliate.

**Incidence and Transmission.**—*B. coli* is widely distributed in most parts of the world but is more common in countries with a temperate climate. However, while the incidence of infection in pigs is high, the occurrence of this ciliate in human beings is relatively rare. Thus, only one or two isolated cases have been reported from England, though balantidiosis of pigs is widespread in this country. Moreover, infections with *B. coli* are usually found in persons who come in close contact with pigs or their carcasses, e.g. pig-farmers,

swineherds, slaughterers, etc. Transmission to man is effected by consumption of food or drink contaminated with the faeces of infected pigs or by direct transfer of cysts to the mouth by hands soiled while handling these animals in piggeries or in slaughter-houses. It would thus seem that balantidiosis is a typical zoonosis, the pig being the reservoir host from which man acquires his infection. It is also possible that the infection is transmitted by human carriers who are passing cysts of *B. coli* in their stools.

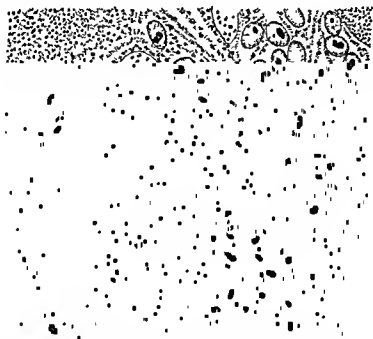


FIG. 17.—BALANTIDIAL ULCER OF THE LARGE INTESTINE OF MAN ( $\times$  ca. 85). (Adapted from Wenyon, 1926.)  
Group of ciliates (*Balantidium coli*) in the submucosa.

**Pathogenesis.**—Balantidial infection of pigs is invariably symptomless, the ciliates living as harmless commensals in the lumen of the gut and feeding on its contents, especially on starch which apparently forms an important part of its diet. In man *B. coli* occurs in the large intestine, in most cases living in the lumen, where it feeds on the contents of the gut, without causing any symptoms of disease. In other cases diarrhoea may be present, while some cases develop an acute form of dysentery. In such cases the ciliates penetrate the epithelial lining of the intestine and invade the sub-

mucous tissues, giving rise to ulcers resembling those produced by *Entamoeba histolytica*. In and around these ulcers the ciliates may be found in large numbers (Fig. 17), multiplying and feeding on red blood corpuscles and on elements of the host's tissues. This condition is manifested by symptoms of diarrhoea or dysentery (balantidial dysentery). It has been suggested that the harmlessness of *Balantidium* in some cases of human infection and its pathological effect in others are due to differences in the host's diet. Since this ciliate feeds mainly on starch it thrives best in the intestine of the pig, where it finds abundant and suitable food. Judging from the fact that balantidial infection is relatively rare in man, it would appear that the conditions in the human bowel are not very favourable to the existence of the ciliate, probably owing to the scarcity of starch. It is thought that insufficiency of suitable food in the lumen of the gut induces the ciliate to attack its walls, with the result that the pathological symptoms of human balantidiosis are produced.

**Occurrence in Lower Mammals.**—In addition to the pig, which is the normal host of *B. coli*, natural infections with this ciliate occur in various monkeys and have also been reported from rats. While it is possible that in some countries monkeys might serve as reservoir hosts, the rôle of rats is not clear, but it is conceivable that they may act as vehicles in the dissemination of cysts.

### DIAGNOSIS

The diagnosis of balantidiosis is based on the detection of *B. coli* in the stools of the patients. The active ciliates are usually found in loose or diarrhoeic stools, in which they can be recognized by their large size, by the characteristic vibrations of the numerous cilia covering their body, and by the rhythmic pulsation of the contractile vacuoles. In formed stools, as a rule, only cysts are found. These appear as large thick-walled spherical or ovoid bodies, in which the macronucleus can be discerned. The methods of preparation and examination of faeces are the same as for other intestinal protozoa (see Chapter 16). Permanent stained preparations can be made by the usual methods (see Chapter 18) but the staining of ciliates is not always satisfactory. The ciliates can also be preserved by mixing the faeces with 5 per cent. formalin, in which they can be kept indefinitely. Their appearance in formalin is similar to that in fresh preparations.

## CHAPTER 8

### THE COPROZOIC PROTOZOA

AMONG the free-living protozoa there are numerous species, belonging to different classes, which show a special predilection for habitats rich in decomposing organic matter, such as soil, stagnant and foul water, mud or sewage. In these media the protozoa find an abundant supply of food in the form of bacteria and other microorganisms, as well as dissolved substances which are absorbed saprozoically. Under natural conditions protozoa of this type are frequently encountered in sewage or in dung infusions, and they grow readily in artificial media to which faecal matter has been added. Such protozoa, which are known as COPROZOIC or COPROPHILIC forms, may also occur in human faeces and their presence in stools has frequently led to confusion. Thus, inexperienced workers are liable to mistake them for known intestinal parasites, while others have described coprozoic forms as new human parasites and have even attributed to them various pathological conditions observed in the patient. The protozoological literature abounds with examples of such misinterpretation.

Coprozoic protozoa may contaminate human faeces in two ways, directly or indirectly. In the first case, they find their way into the stool after it has been discharged: if conditions are favourable the deposited faeces may provide a suitable culture medium in which the protozoa live and multiply as they do in any other decaying organic infusion. In the second case, the cysts of coprozoic protozoa—which may be air-borne or water-borne—are swallowed with food or drink contaminated by them: in such cases the cysts may pass unharmed through the alimentary canal and be discharged in the faeces, in which they hatch and multiply as in the first case. Unlike true parasites, coprozoic protozoa are incapable of living at the temperature of the human body, but develop normally at laboratory temperature.

In practice, contamination of human stools with coprozoic protozoa may take place if faecal examination is delayed for some reason, and especially if the stool is diluted with water or saline. While tap-water is a natural source of free-living protozoa, they are



also liable to develop in distilled water or in saline, if these have been stored for some time. Even if the stools have not been deliberately diluted, protozoa may gain access to them from bed-pans, when these are used undried after having been flushed with water.

The development of extraneous protozoa in stools can be prevented by using perfectly dry or even sterilized containers, by avoiding their dilution, unless freshly boiled water or saline is used, and finally by examining the faeces immediately after they have been passed. However, if none of these precautions has been taken, the coprozoic nature of protozoa found in the faeces can be established by cultivating a portion of the faeces containing them at room temperature in nutrient fluid media or on agar plates, to which some sterile water has been added. While parasitic protozoa rapidly die out in such media at temperatures below 37° C., coprozoic protozoa develop normally and can be maintained indefinitely by serial passages of cultures at room temperature.

Coprozoic protozoa are common among the Rhizopoda, Mastigophora and Ciliophora, some of them having until quite recently figured as human intestinal parasites. A brief description will be given of some of the most important species which may be encountered in human stools.

#### (a) AMŒBÆ

##### *Hartmannella hyalina* (Dangeard, 1900)

*Hartmannella hyalina* (Fig. 18, c) occurs naturally in damp soil and in water; it is frequently found in stale faeces and can be cultivated on agar plates. When rounded, the amœba measures 5-17 $\mu$  in diameter. It can be distinguished from the human intestinal amœbæ by the structure of its nucleus, which is of the vesicular type, with a large central karyosome and numerous chromatin granules in the space between the karyosome and the nuclear membrane. It also possesses a single contractile vacuole, a structure which is absent in parasitic amœbæ. *Hartmannella* produces uninucleate cysts, measuring 10-14 $\mu$  in diameter, with a characteristic thick wrinkled wall.

##### *Dimastigamœba gruberi* (Schardinger, 1899)

*Dimastigamœba gruberi* (Fig. 18, a, b) is a common free-living amœba, living in water and in soil, and frequently occurring in

stale faeces of man and other animals. This organism occupies an intermediate position between the amœbæ and flagellates. It usually has the appearance of a typical amœba, measuring 7-15 $\mu$  in diameter, when rounded. The single vesicular nucleus has a

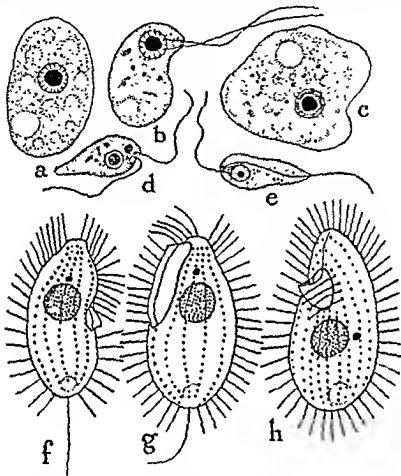


FIG. 18.—COPROZOIC PROTOZOA ( $\times 2,000$ ). (Adapted from various authors.)

a, b. *Dimastigamæba gruberi*: amœboid and flagellate forms; c. *Hartmannella hyalina*; d. *Bodo caudatus*; e. *Cercomonas longicauda*; f. *Uronema nigricans*; g. *Lembus pusillus*; h. *Balantiothorus minutus*.

large central karyosome connected by fine radial filaments with the nuclear membrane, which bears a small number of chromatin granules (peripheral chromatin). There is a single contractile vacuole. Under certain conditions, due to changes in the environment, the amœba becomes elongated and pear-shaped, its nucleus

moves to the narrower anterior end and two long flagella develop from basal granules at the anterior pole of the nucleus, the organism thus assuming a flagellate stage. The two stages, amœboid and flagellate, are reversible. *Dimastigamœba* produces spherical uninucleate cysts, measuring on the average  $10\mu$  in diameter. They have a thick wall in which there are several pores, through one of which the amœba emerges when hatching.

### (b) FLAGELLATES

#### *Bodo caudatus* (Dujardin, 1841)

*Bodo caudatus* (Fig. 18, d) lives in stagnant water and in various organic infusions. It is readily cultivated in hay infusion and on agar plates. It sometimes appears in water or saline stored in the laboratory and when these are used for staining purposes the presence of *Bodo* in the preparations may lead to confusion. This flagellate is a common coprozoic form, which has occasionally been mistaken for and described as a true intestinal parasite of man.

The body of this flagellate is rounded or elongated and slightly flattened, the posterior end being sometimes pointed. Its length varies from 11 to  $18\mu$ . At the anterior end there is a slight prominence with a lateral depression, representing the mouth (cytostome), and a small contractile vacuole. Near these structures there is a rounded deeply-stainable body, the kinetoplast, which probably plays some rôle in the metabolism of the organism and corresponds to a similar structure present in leishmanias and trypanosomes. From a pair of basal granules on the surface of the kinetoplast there arise two long flagella, one of which is directed forwards, while the other trails backwards. Food-vacuoles, containing bacteria, are usually found in the hind end of the body. *Bodo* produces small oval cysts with thin walls, measuring  $5-7\mu$  in length.

#### *Cercomonas longicauda* Dujardin, 1841

*Cercomonas longicauda* (Fig. 18, c) commonly occurs in various infusions and may lead a coprozoic existence in the faeces of various animals, including man. Like the other coprozoic protozoa it readily grows in cultures.

The body of this flagellate is pear-shaped but is capable of metabolic changes of form, when it becomes amœboid. Its length

varies from 5 to 18 $\mu$ . There is no mouth opening, food being taken in through the surface of the body, as in amœbæ. The nucleus, containing a large central karyosome, is situated in the anterior end of the body, its anterior pole being conical with the apex directed forwards. From minute basal granules (blepharoplasts) at the apex of the cone two long flagella arise. One of these is free and directed forwards, while the other runs backwards and is attached for the greater part of its length to the surface of the body; on reaching the hind end of the body it emerges as a short free flagellum. *Cercomonas* forms small spherical cysts, measuring 6-7 $\mu$  in diameter.

### (c) CILIATES

Medical literature contains a number of records of coprozoic ciliates found in human stools and associated with various pathological conditions of the patient. In many cases these ciliates were regarded as human intestinal parasites and were described under different names, the most notorious being "*Uronema caudatum*" and "*Balantidium minutum*." Subsequent work has shown that these ciliates were actually coprozoic forms which had gained access to the stools, and these have provided a suitable culture medium for their growth. There is, however, some doubt regarding the identity of some of these coprozoic forms. The pseudo-parasitic ciliates described by different authors under the name "*Uronema caudatum*" probably represented two or three distinct species, *Cyclidium glaucoma*, *Uronema nigricans* and *Lembus pusillus*. As regards "*Balantidium minutum*," which has figured for the last half-century as the second species of *Balantidium* parasitic in man, it has recently been identified with *Balantiophorus minutus*. All these ciliates are free-living forms showing a special predilection for habitats rich in organic matter and occurring in stagnant water, in sewage and in soil, in which they feed on bacteria. They thrive in faecal infusions, in which they are readily cultivated, and are also found in the stools of man and other animals. A description will now be given of three species of closely related ciliates, the coprozoic habits of which have been more thoroughly studied.

#### *Uronema nigricans* (Müller, 1786)

*Uronema nigricans* (Fig. 18, f) occurs both in fresh and salt waters, where it lives on the surface amongst decomposing matter.

It has also been found in sewage. This ciliate has an elongated body, narrowing towards the anterior end, which is slightly bent to one side and obliquely truncated. Its length varies from  $19\mu$  to  $32\mu$ . The body is covered uniformly with cilia; its posterior end bears a long caudal cilium, or seta. In the middle of the body there is a slight depression (peristome) in which the mouth (cytostome), carrying a small undulating membrane, is situated. The macronucleus and adjacent micronucleus are in the middle of the body, and there is a contractile vacuole in the posterior end.

*Lembus pusillus* Quennerstedt, 1869

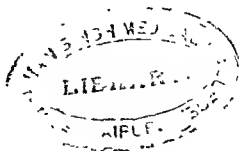
*Lembus pusillus* (Fig. 18, g) has the same habits as *Uronema nigricans*, its natural habitat being sea water and fresh water, where it lives among decaying organic matter, showing a special predilection for foul water and faecal matter. The body is elongated ovoid, gradually narrowing anteriorly, the anterior end being slightly bent to one side. The ciliate measures  $24-37\mu$  in length. It is covered with twelve rows of long cilia and has a very long caudal cilium, or seta, protruding from the posterior end of the body. There is a large peristomial depression, which starts near the anterior end and extends beyond the middle of the body, where it is prolonged in the form of a funnel-shaped invagination leading to the mouth (cytostome). The right border of the peristome bears two long undulating membranes which serve to drive the food into the mouth. The spherical macronucleus, accompanied by a micronucleus, is situated in the anterior third of the body. In the hind end there is a contractile vacuole.

*Balantiophorus minutus* Schewiakoff, 1889

*Balantiophorus minutus* (Fig. 18, h) occurs in foul waters and in soils rich in decaying organic matter, being especially common in habitats contaminated with animal faeces, where it finds abundant food in the form of bacteria. As mentioned already, this coprophilic ciliate is of particular medical interest, since it has been mistaken for an intestinal parasite of man, masquerading for many years under the name "*Balantidium minutum*."

The body of *B. minutus* is ovoid, with a narrower anterior end, which is slightly bent. Its length is variable, the majority of ciliates measuring from  $25\mu$  to  $45\mu$ . The body is uniformly covered with

cilia. In the middle of the anterior half of the body is a small peristomial depression with a funnel-like posterior extension leading to the mouth (cytostome). The borders of the peristome carry an undulating membrane in the form of a hood open in the front. The macronucleus, with an embedded micronucleus, is situated in the middle of the body. Near the hind end of the body lies a large contractile vacuole.





## SECTION B

### PROTOZOA OF THE BLOOD AND OF THE RETICULO-ENDOTHELIAL SYSTEM

SOME of the most important protozoal diseases of man are caused by parasites which inhabit the blood and various fixed tissues, especially those belonging to or containing elements of the reticulo-endothelial system, also known as the lymphoid-macrophage system. These protozoa are responsible for Malaria, Sleeping Sickness, Chagas' Disease, Kala-Azar, Oriental Sore and Toxoplasmosis.

Some of these parasites, viz. trypanosomes, are actively motile and live freely in the blood stream and in certain other body fluids (e.g. *Trypanosoma gambiense* and *T. rhodesiense*), or they may invade various tissue-cells at some period of their life-cycle and lead an intracellular existence (e.g. *T. cruzi*). Others are exclusively intracellular parasites, living either within the erythrocytes (e.g. the malaria parasites, genus *Plasmodium*) or within cells of the reticulo-endothelial system (e.g. *Leishmania*, *Toxoplasma*).

A peculiar feature of most of these blood- and tissue-inhabiting protozoa is their relation to the reticulo-endothelial system. As is known, the reticulo-endothelial system is represented by fixed and wandering cells, commonly known as macrophages, which are widely distributed in various tissues of the body, being particularly abundant in the blood-forming and blood-destroying organs. Thus, they are common in the spleen, bone-marrow, lymph glands and liver, as well as in the loose connective tissue, heart, intestinal villi, central nervous system and in serous cavities of the body. The wandering cells are also present in the circulating blood. The macrophages play an important part in the defence mechanism of the host, by actively phagocytosing dead and living particulate matter (cellular reaction) and by elaborating antibodies (humoral reaction).

While in Sleeping Sickness the reticulo-endothelium appears to exercise its normal functions fully, by immobilizing or phagocytosing the trypanosomes, in other protozoal infections of the blood the parasites are able to a greater or less degree to resist the phagocytic action of the macrophages. An example of such adaptation is provided by *Trypanosoma cruzi* which is capable of developing within the cells of the reticulo-endothelial system, a property





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apparently shared by *Toxoplasma*, while the leishmanias afford an instance of complete adaptation, for they are normally parasitic within the reticulo-endothelial cells exclusively. It is thus seen that some of the blood parasites live and multiply in the very phagocytes which in other diseases represent the principal means of defence.

Unlike the intestinal protozoa, in which the entire life-cycle is restricted to the human host and transmission to a new host depends upon accidental ingestion of the cysts deposited in the stool, the blood-inhabiting protozoa have two hosts, man and a blood-sucking insect, the latter serving as vector in the transmission of the infection. In some of these parasites (*Trypanosoma cruzi*) transmission is contaminative, infection depending upon the infective stages being accidentally deposited on vulnerable parts of the body, while in others (*T. gambiense*, *T. rhodesiense* and the malaria parasites) the success of infection is ensured by direct inoculation of the infective stages into the human host.

A list of the protozoa inhabiting the blood and reticulo-endothelial system is given in Table 5. These parasites belong to the classes Mastigophora and Sporozoa, the main characteristics of which have already been described. A detailed account of the species named in the table is given in the chapters which follow.

TABLE 5  
PROTOZOA OF THE BLOOD AND OF THE RETICULO-  
ENDOTHELIAL SYSTEM

CLASS	COLLECTIVE NAME		GENUS	SPECIES	HABITAT
MASTIGOPHORA	Hæmoflagellates	Leishmanias	<i>Leishmania</i>	<i>donovani tropica</i>	Reticulo-endothelial system
		Trypanosomes	<i>Trypanosoma</i>	<i>gambiense rhodesiense</i>	Blood, lymph glands, cerebrospinal fluid
				<i>cruzi</i>	Blood, muscles, reticulo-endothelial system
SPOROZOA	Malaria parasites		<i>Plasmodium</i>	<i>falciparum vivax malarie ovale</i>	Blood, reticulo-endothelial cells (?), liver
	<i>Toxoplasms</i>		<i>Toxoplasma</i>	<i>hominis</i>	Brain and other tissues

## CHAPTER 9

### THE HÆMOFLAGELLATES

THE Mastigophora living in the blood and/or the reticulo-endothelial system and some other tissues of man, and commonly known as the Hæmoflagellates, are represented by six species belonging to two genera, *Leishmania* and *Trypanosoma*. The leishmanias and trypanosomes are very closely related to each other zoologically and have essentially the same structure, differing mainly in the arrangement of the different cytological elements.

As an example, which—with modifications—can serve for both hæmoflagellates, we may take the structure of a typical trypanosome (Fig. 19). The trypanosome has an elongated body which is flattened and somewhat lancet-shaped. It has a single nucleus of the vesicular type containing a large central karyosome (Fig. 19, a). This structure is revealed only in preparations stained after "wet"

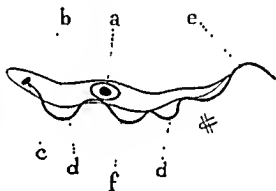


FIG. 19.—STRUCTURE OF TRYPANOSOME.  
(After Hoare, 1940.)

a. Nucleus; b. Kinetoplast; c. Basal granule (blepharoplast); d, e. Flagellum (marginal and free portions); f. Undulating membrane.

fixation but in those fixed by the "dry" method and stained by one of the Romanovsky methods the nucleus appears to be evenly filled with irregular granules (Pl. II, e-i). The organ of locomotion is represented by a single flagellum (Fig. 19, d, e) which starts at one end of the body and runs to the opposite end along the outer margin of a frilled outgrowth of the pellicle, which extends throughout the length of the body and is known as the undulating membrane (Fig. 19, f). When the flagellum reaches the end of the membrane, it either becomes free (Fig. 19, e) or terminates at the end of the membrane without a free portion (Fig. 25, c). In the former case

we speak of trypanosomes possessing a *free* flagellum, in the latter case of those without a free flagellum. It has already been stated (Chapter 5) that in Mastigophora the end of the body from which the flagella emerge freely is regarded as the anterior end. Therefore, in trypanosomes the flagellum starts from the posterior end of the body and terminates in the anterior end. As in other Mastigophora, the flagellum arises from a minute basal granule, or blepharoplast (Fig. 19, c), which is usually inconspicuous. At the starting point of the flagellum there is also a prominent chromatin-staining structure, the KINETOPLAST (or kinetonucleus) (Fig. 19, b). This structure, which is characteristic of the Hæmosflagellates, is disc-shaped but it may appear to be rounded (Pl. II, i, k, r, s) or rod-shaped (Fig. 19, b), according to its orientation in the body of the flagellate. The exact function of the kinetoplast is not known but it is believed to play some part in the metabolism of glucose, which is consumed by the Hæmosflagellates from the blood of the host.

Multiplication in the human Hæmosflagellates is by binary longitudinal fission, which can also be illustrated by the division of a trypanosome. This proceeds as follows (Fig. 20): first, the kinetoplast and the basal granule divide into two; one of the daughter-kinetoplasts retains the old flagellum, while a new one grows out from the basal granule accompanying the second kinetoplast (Fig. 20, b); in the meantime the nucleus also divides into two, while the new flagellum increases in length until it is as long as the old one (Fig. 20, c, d); after the trypanosome has developed a double set of nuclei and other organs the body itself undergoes longitudinal fission, which begins from the anterior end (Fig. 20, e), until finally the two daughter-trypanosomes become separated.

The Hæmosflagellates do not possess a mouth opening but absorb food in dissolved form through the entire surface of the body. There is no satisfactory evidence of the occurrence of sexual phenomena in any of the Hæmosflagellates.

Both leishmanias and trypanosomes undergo a cycle of development with an alternation of hosts. One of these is man (and in some cases other mammals), who is regarded as the final host, while a blood-sucking insect represents the intermediate host, or vector, which serves to transmit the infection.

In the course of their life-cycle in both hosts the Hæmo-

flagellates pass through a number of stages which are distinguished by a definite type of structure. There are altogether four morphological types or stages of development which, in different combinations, serve as criteria for the determination of the genus to which a hæmoflagellate belongs (Fig. 21).

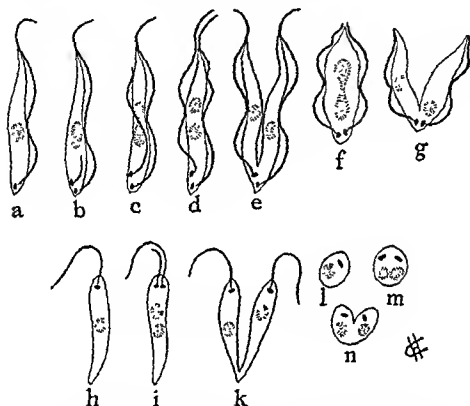


FIG. 20.—DIVISION OF HÆMOFLAGELLATES. (Original.)

a-g, Division of trypanosomes: a, Non-dividing form; b, Kinetoplast

These stages are as follows :—

(1) The trypanosome stage (already described) has an elongated body; the kinetoplast and the starting point of the flagellum are situated in the posterior part of the body, behind the nucleus (postnuclear);

(2) The crithidial stage has an elongated body; the kinetoplast

and the starting point of the flagellum are situated in the middle of the body, near the nucleus (juxtannuclear);

(3) The leptomonad stage has an elongated body; the kinetoplast and the starting point of the flagellum are situated in the anterior end of the body, in front of the nucleus (antennuclear);

(4) The leishmanial stage has a rounded body; the flagellum is absent, but the kinetoplast is present.

All these stages, except the last, can be regarded as a modification of the trypanosome type by gradual displacement forwards of the

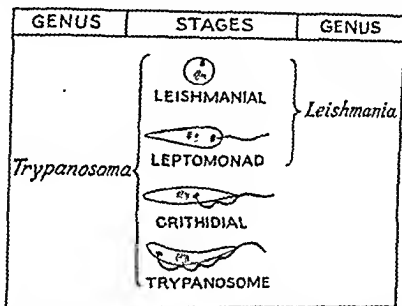


FIG. 21.—CLASSIFICATION OF THE GENERA OF HÆMOFLAGELLATES ACCORDING TO THEIR STAGES OF DEVELOPMENT. (Original.)

starting point of the flagellum and the kinetoplast. As regards the leishmanial stage, it may arise from any of the preceding stages by loss of the flagellum and rounding of the body. By reversing the process, i.e. by developing a new flagellum and becoming elongated, the leishmanial form may revert to a flagellate form of one of the above stages. While in general the leishmanial form may be assumed by any of the hæmoflagellates under unfavourable conditions and by degenerating individuals, in some (e.g. *Leishmania*, *Trypanosoma cruzi*) it represents a constant stage of development normally occurring in the course of their life-cycle.

The Hæmoflagellates comprise two genera, belonging to the family Trypanosomidæ, *Leishmania* and *Trypanosoma*, species of

which are parasitic in all classes of vertebrates and include the human parasites causing various forms of leishmaniasis (Kala-Azar, Oriental Sore) and trypanosomiasis (Sleeping Sickness, Chagas' Disease). These flagellates are DIGENETIC, their life-cycle involving an alternation of hosts, the vertebrate and invertebrate. The latter serves as vector which transmits the active flagellates from one vertebrate host to another.

In the genus *Leishmania* there are only two stages of development, the leishmanial and leptomonad stages, while members of the genus *Trypanosoma* pass through all the above-named stages—trypanosome, and leishmanial—in the course of their

In addition to these two genera, which are the only ones of medical interest, the family Trypanosomidae comprises the genera *Crithidia*, *Herpetomonas*, *Leptomonas* and *Phytomonas*. Members of the first three genera are MONOGENETIC intestinal parasites, the life-cycle of which is restricted to one host only, represented by invertebrate animals, especially insects. The transmission of these flagellates is effected by cysts of the leishmanial type, which are voided in the droppings of the host, a new host being infected by swallowing these cysts, as in the case of human amœbiasis. Flagellates of the last-named genus, *Phytomonas*, are digenetic parasites of plants and insects. They live in the latex of certain plants (e.g. milkweeds) and are transmitted by bugs feeding on these plants.





## CHAPTER 10

### THE LEISHMANIAS

#### GENUS *LEISHMANIA* ROSS, 1903

FLAGELLATES of the genus *Leishmania*, commonly known as leishmanias, are parasitic in man, dog, gerbils and some other mammals, which represent the final hosts, while sandflies (blood-sucking Diptera of the genus *Phlebotomus*) serve as intermediate hosts, or vectors, which transmit the infection from one mammal to another. In man and dog these parasites cause a number of diseases, known collectively as LEISHMANIASES, which are of two main types: (1) Visceral leishmaniasis, or Kala-Azar, and (2) Cutaneous leishmaniasis, better known as Oriental Sore, and including a variant known as mucocutaneous leishmaniasis, or Espundia.

#### MORPHOLOGY AND LIFE-HISTORY

In the course of their life-cycle in the mammalian and insect hosts these parasites pass through two stages of development—the rounded leishmanial form and the flagellate leptomonad form (Fig. 21). In the human body and in that of other mammalian hosts the parasites occur only in the leishmanial stage, whereas the leptomonad stage is assumed by them in the insect-vector and in cultures.

There is no morphological difference between the various species of *Leishmania* of man and of other mammals, therefore they need not be described separately.

#### The Parasite in the Mammalian Host

When seen in the mammalian host the parasites are small ovoid or round bodies (sometimes described as "Leishman-Donovan bodies"), measuring  $2-5\mu$  by  $1.5-2.5\mu$ , when ovoid (Pl. II, b; Fig. 22, b). The only inner structures usually visible are the nucleus and the kinetoplast. Though their relative positions may vary, the nucleus frequently lies against one side of the body, while the kinetoplast appears in the form of a rod usually lying transversely with its long axis towards the nucleus. In preparations stained by one of the Romanovsky methods, the nucleus appears as a red-coloured rounded granular mass and the kinetoplast as a dark

ruby-coloured rod or granule. The recognition of these two elements in the rounded parasite is of the greatest diagnostic value in leishmaniasis. Occasionally there can be seen the rudiment of a flagellum, or axoneme, in the form of a short filament arising from the kinetoplast. In preparations fixed and stained by the "wet" method the nucleus reveals a typical vesicular structure, as in trypanosomes (Fig. 19). In stained sections of infected tissues the

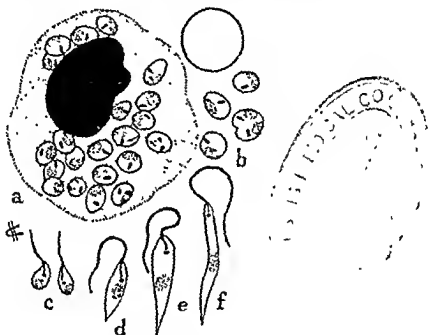


FIG. 22.—*Leishmania donovani* AND *L. tropica* ( $\times 2,000$ ). (After Hoare, from Broom, 1942.)

a. Macrophage containing rounded parasites ("Leishman-Donovan bodies"); b. Parasites outside host-cell (one dividing); c-f. Flagellate (leptomonad) forms as seen in sandfly and in cultures. Erythrocyte drawn to scale.

kinetoplast is sometimes inconspicuous, the parasites having the appearance of cocci.

In the mammalian host the leishmanias are intracellular parasites exclusively, being enclosed within the cytoplasm of macrophages in tissues containing elements of the reticulo-endothelial system (Pl. II, a; Fig. 22, a), but in stained tissue smears the parasites are frequently seen outside the host-cells (Pl. II, b; Fig. 22, b) or in non-nucleated fragments of these (Pl. II, c). Such cases are, however, due to artificial breaking-up of these cells in the course of prepara-

tion. In sections of infected tissues—where the host-cells remain intact—the parasites are invariably intracellular.

**Reproduction.**—*Leishmanias* multiply exclusively by binary fission (Fig. 20, l-n). First the kinetoplast elongates and divides into two parts. This is followed by division of the nucleus and finally by fission of the body, giving rise to two daughter-individuals. After repeated binary fissions of the intracellular parasites the host-cells containing them may become packed with leishmanial forms, which can be distinguished individually (Pl. II, a ; Fig. 22, a), though in imperfectly stained dry preparations they sometimes fuse and the outlines of the individual parasites become invisible. In such cases the parasites within the host-cell have the appearance of a single body with numerous nuclei and kinetoplasts. This picture has been misinterpreted by some observers as multiple division, or schizogony, in *Leishmania*.

The course of development of leishmanias in the macrophages has not been fully elucidated. It is thought that after a period of multiplication the host-cell, which is enlarged and filled with parasites, bursts or disintegrates, releasing the parasites. It is doubtful whether the rounded leishmanias are capable of actively invading new host-cells ; it is more probable that the macrophages, in their phagocytic capacity, engulf the parasites, which then start multiplying within the new cell.

**Cultivation.**—The leishmanias are readily cultivated in various artificial media (see Chapter 19) at temperatures between 22° C. and 25° C., i.e. approximately at temperatures at which their sandfly vector lives, the development of the parasite in culture being comparable to its development in the intermediate host (see below). In culture the leishmanial stage of the parasite, inoculated with the host's tissues or blood, is transformed into the flagellate leptomonad stage. In the course of this development a flagellum starts growing from the basal granule near the kinetoplast, the body of the rounded parasite gradually elongates and becomes spindle-shaped, while the kinetoplast assumes a position in the anterior end of the body from which a long free flagellum is given off (Fig. 22, c-f). Under favourable conditions leishmanias can be maintained in cultures, by serial subinoculation, for an almost indefinite period, the leptomonad forms multiplying by binary fission in the same way as trypanosomes (Fig. 20, h-k).

## The Parasite in the Insect-Host

The intermediate hosts, or vectors, of *Leishmania* are sandflies, Diptera of the genus *Phlebotomus* (Fig. 23) the females of which are blood-sucking. The different forms of leishmaniasis are transmitted by various species of these insects.

When a sandfly feeds on an infected human being or other mammalian host, the leishmanias, ingested together with the blood, are taken into the midgut, or stomach, of the insect (Fig. 24, f). Here they proceed to develop in the same manner as they do in the culture-tube (see above), i.e. they become leptomonad flagellates (Fig. 22, c-f) which multiply rapidly until by the third day there are masses of them in the midgut, where they are attached to the walls and lie free in the lumen. The flagellates are especially numerous in the anterior region of the midgut, or proventriculus (Fig. 24, e). As they increase in numbers the flagellates begin to spread forwards and on the fourth or fifth day they ascend into the oesophagus and pharynx (Fig. 24, d, e, b). Gradually they fill this region so densely that the passage is blocked by them, producing a condition similar to that in fleas infected with plague bacilli.



FIG. 23.—SANDFLY (*Phlebotomus*): VECTOR OF LEISHMANIASIS. (From Wellcome Museum of Medical Science, London.)

**Transmission.**—When an infected sandfly bites a new host and attempts to feed, the plug of flagellates interferes with the flow of blood. Its strenuous efforts to obtain a meal cause some of the flagellates to become dislodged from the buccal cavity, with the result that they are injected through the proboscis into the skin together with the saliva and give rise to infection. There is no evidence of a special infective stage among the leptomonad flagellates, which are indistinguishable morphologically, though differing in size (Fig. 22, c-f). The method of dissection of sandflies for the detection of the developmental stages of leishmanias is described in Chapter 20.

It is conceivable that transmission might also be effected by crushing the sandflies on the skin: if there is an abrasion, the

liberated flagellates are able to penetrate into the body of the host.

After having been introduced into the skin of the mammalian host through the bite of the sandfly, the flagellates become rounded and assume the leishmanial form. They are then taken up by macrophages of the subcutaneous tissues in which they multiply until the host-cell ruptures and the infection spreads locally among cells of the reticulo-endothelial system. The reticulo-endothelial cells of the skin thus represent the primary site of a leishmanial infection, irrespective of whether it develops into the visceral or cutaneous form of the disease. The only difference in the subsequent course of the infection is that in visceral leishmaniasis the invaded macrophages enter the blood circulation and are carried to the inner organs, where they give rise to a generalized infection, whereas in cutaneous leishmaniasis the infection does not spread beyond the subcutaneous tissues, where it remains localized.

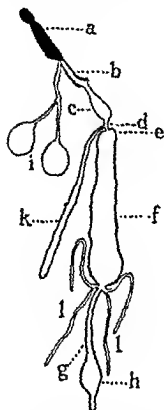


FIG. 24.—ALIMENTARY TRACT OF SANDFLY.

(Adapted from Wenyon, 1932.)

a. Proboscis; b. Pharynx; c. Esophageal pump; d. Esophagus; e. Proventriculus; f. Midgut; g. Hindgut with rectum (h); i. Salivary glands; k. Esophageal diverticulum; l. Malpighian tubes.

lesion usually escapes notice and is, therefore, of little practical value in diagnosis.

## CLASSIFICATION

Human leishmaniasis occurs both in the Old and in the New World, in which the causative organisms have been given different names.

In the Old World there are two recognized species of the genus *Leishmania*: (1) *L. donovani*, causing visceral leishmaniasis or Kala-Azar, and (2) *L. tropica*, causing cutaneous leishmaniasis or Oriental Sore.

Some authors have differentiated from *L. donovani* another species, *L. infantum*, for the parasite causing infantile leishmaniasis in the Mediterranean area, but there is no justification for its separation. Distinct names have also been given to the parasites of canine leishmaniasis, viz. *L. canis* for the one causing visceral leishmaniasis in dogs and *L. tropica* var. *canina* for that causing cutaneous leishmaniasis, but the retention of these names is likewise unjustified, for we now have experimental evidence of the identity of the canine and human parasites, based on cross-infection of their hosts.

Cutaneous and visceral human leishmaniasis also occur in Central and South America, the parasites there being known as *L. brasiliensis* and *L. chagasi* respectively. Though the disease caused by *L. brasiliensis* may develop into a special form, known as mucocutaneous leishmaniasis, in most cases the course of cutaneous leishmaniasis in the New World is similar to that in the Old World, therefore it would seem that *L. brasiliensis* represents merely a variant of *L. tropica* and is specifically identical with the latter species. As regards visceral leishmaniasis, the clinical course of the infection caused by *L. chagasi* and by *L. donovani* is the same, on account of which the latter name should be retained for the parasite in all parts of the world.

It has already been stated that the parasites causing various types of leishmaniasis are morphologically indistinguishable. Attempts have also been made to differentiate the various species of *Leishmania* by biological methods, viz. (a) by their growth in culture; (b) by fermentation reactions; and (c) by serological methods. However, the results obtained by different workers are conflicting and there is no conclusive evidence that the species of *Leishmania* can be identified by any of these methods. In practice the only clue to the species of parasite is provided by the clinical picture of the case from which it was obtained. However, in the case of dogs even this criterion may fail, as will be shown below.

There is, therefore, every reason to believe that the various clinical manifestations in cutaneous and visceral leishmaniasis are

actually due to biological races of one and the same species of *Leishmania*, but for practical convenience the parasites will be referred to under the existing specific names.

The accepted classification is summarized in Table 6 which gives both the valid names of species and their synonyms.

TABLE 6  
SPECIES OF *LEISHMANIA*

TYPE OF LEISHMANIASIS	VALID NAMES	SYNONYMS
Visceral . .	<i>L. donovani</i>	<i>L. infantum</i> , <i>L. chagasi</i> , <i>L. canis</i>
Cutaneous .	<i>L. tropica</i>	<i>L. brasiliensis</i> , <i>L. tropica</i> var. <i>canina</i>

Since the foregoing account of the structure, life-history and bionomics of the leishmanias applies to both *L. donovani* and *L. tropica*, the succeeding sections devoted to these species will deal mainly with their host-parasite relationships and diagnosis.

#### xvii. *LEISHMANIA DONOVANI* (LAVERAN & MESNIL, 1903)

**Relation to Disease.**—*Leishmania donovani* is the cause of visceral leishmaniasis or Kala-Azar, a disease characterized by a general invasion by the parasites of the reticulo-endothelial system in various organs and manifested chiefly by enlargement of the spleen and liver, anæmia and irregular fever.

**Geographical Distribution.**—Kala-Azar is widely but discontinuously distributed in warm countries. In the Old World it occurs in China, India, Soviet Central Asia, in countries of the Middle and Near East, in Arabia and the Anglo-Egyptian Sudan, in the Caucasus and in countries bordering on the Mediterranean as far as Portugal in the West. In the New World the disease is restricted to parts of South America.

#### HOST-PARASITE RELATIONSHIP

**Types of Disease.**—Visceral leishmaniasis is a disease affecting both human beings and dogs. It appears in three main forms:

(1) The Indian form of Kala-Azar, which affects mainly children between the ages of 5 and 15 years, and young adults (60 per cent.) but is not found in local dogs. This is the classical type of the disease, as it appears in India. (2) Infantile Kala-Azar, which affects chiefly young children under the age of 5 (90 per cent.) as well as dogs. Infantile Kala-Azar occurs in China, Central Asia, countries of the Mediterranean basin (all North Africa, Balkan countries, Italy, Malta, southern France, the Iberian Peninsula) and in some parts of tropical Africa. South American visceral leishmaniasis also belongs to this type. (3) Sudanese Kala-Azar, which chiefly affects the same age-groups as the Indian type and likewise does not occur in local dogs. This type of Kala-Azar occurs in the Anglo-Egyptian Sudan and in Abyssinia, and is characterized by the frequency of skin infections, with or without visceral involvement, and by the occurrence of mucocutaneous lesions.

**Course of Infection.**—At the site of a bite by an infected sandfly there appears on the skin a small papule, which gradually increases in size and disappears several months later. The parasites are first restricted to these primary lesions, in scrapings of which they can be detected before the appearance of clinical symptoms. Later infected macrophages enter the blood stream and are carried from the site of initial infection to various internal organs, where they invade cells of the reticulo-endothelial system in which they multiply intensively. The parasites occur wherever there are macrophages, being especially abundant in the spleen, liver, intestinal submucosa, bone-marrow and lymph glands, as well as in the blood where they may be found within wandering macrophages and monocytes. The invasion of the reticulo-endothelium by the parasites causes not only enlargement of the infected cells but also increased proliferation of other macrophages in the vicinity, resulting in considerable enlargement of the organs which are chiefly affected, such as the spleen and the liver. The pathological effect of Kala-Azar is due to blockade of the reticulo-endothelial system, to progressive anaemia resulting from invasion of the bone-marrow, and to chronic intoxication of the organism by the parasites.

The incubation period of Kala-Azar is several months, when the disease can be recognized clinically.

In addition to the inner organs, the parasites may also be present in the skin of infected persons. Cutaneous infection is especially



common in the Sudan and has also been recorded from China and Central Asia. In about 60 per cent. of cases examined in the Sudan a cutaneous infection co-exists with the visceral infection, while in some cases the infection is purely cutaneous, without visceral involvement. It would appear that in India infection of the skin is not apparent during the visceral phase of the disease, but usually reveals itself a year or so after the visceral infection has terminated, spontaneously or as the result of treatment, giving rise to the condition known as *post-kala-azar dermal leishmaniasis* or "dermal leishmanoid." The cutaneous infection is usually manifested by characteristic nodular dermal lesions containing the parasites, which multiply in the macrophages and can be detected in scrapings and cultures from the nodules.

**Immunity.**—There is some evidence of natural and acquired immunity in Kala-Azar. Thus, it has been demonstrated that deliberate injection and accidental inoculation of infected material rarely produce infection in adult human beings, while patients cured of the disease are usually protected against reinfection.

It has been suggested that the difference in the age-incidence of the disease in different endemic areas may be due to the degree of immunity acquired by the population, which is determined by different epidemiological factors. Thus, in the Mediterranean area young children living in close contact with infected dogs are exposed to repeated infections, as the result of which they develop partial immunity or premunity which protects them from infection in later life. On the other hand, in India, where animal reservoirs are unknown, conditions favouring the building up of immunity are absent, therefore older children and adults are fully susceptible to infection. This view finds support in the fact that during the last war cases of Kala-Azar acquired in the Mediterranean region were common among British and American troops coming from non-endemic areas.

### Infection in Lower Mammals

**Canine Visceral Leishmaniasis.**—As already stated, visceral leishmaniasis is also a natural disease of dogs, which is encountered in regions where the Mediterranean (infantile) type of human Kala-Azar is prevalent, but is not found in association with the Indian type of the disease. In natural infections of dogs the parasites occur

both in the internal organs and in the subcutaneous tissues, where they are found within the macrophages. The skin is the chief tissue affected, the parasites being present in nodular lesions or in skin that appears macroscopically to be normal. Sometimes infected dogs show no external symptoms of the disease. Dogs can also be infected experimentally from human cases.

There is no doubt that infantile Kala-Azar of the Mediterranean type and visceral canine leishmaniasis are closely connected. There is a complete parallelism in the geographical distribution, incidence and seasonal occurrence of the two diseases, and it has been demonstrated (in Sicily and in Central Asia) that systematic destruction of infected dogs brought about a marked diminution in the incidence of human infections. The dog is therefore the reservoir host of infantile Kala-Azar and some observers maintain that this disease is a zoonosis, being mainly a disease of dogs, from which it may spread to children. The incidence of infection in dogs may reach 20 per cent. in some countries.

On the other hand, the Indian and Sudanese forms of Kala-Azar appear to be independent of dogs as a source of infection. Numerous examinations carried out in India and involving thousands of these animals failed to reveal the infection in them, though dogs are susceptible to experimental infection with Indian strains of *L. donovani*.

**Experimental Infection.**—In addition to dogs, cats, monkeys, mice and rats are susceptible to experimental infection with *L. donovani*. However, the most suitable laboratory animals are hamsters, rodents of the genus *Cricetus*. The course of infection in these animals is similar to that in man and is accompanied by enlargement of the spleen, which usually contains numerous parasites. Hamsters are used extensively in transmission experiments and for testing leishmanicidal drugs.

### Transmission

Visceral leishmaniasis is transmitted by sandflies, blood-sucking Diptera of the genus *Phlebotomus* (Fig. 23), the species concerned with the transmission of this disease varying in different countries as shown in the accompanying list (Table 7).

The sandfly becomes infected by feeding on exposed parts of the body of the mammalian host. In India man appears to be the

TABLE 7  
CHIEF VECTORS OF VISCERAL  
LEISHMANIASIS

COUNTRIES	SPECIES OF <i>Phlebotomus</i>
China . . .	<i>P. chinensis</i>
India . . .	<i>P. argentipes</i>
Central Asia . . .	<i>P. papatasi</i>
Sudan . . .	<i>P. orientalis</i>
Mediterranean area .	<i>P. perniciosus</i> , <i>P. major</i>
South America. .	<i>P. longipalpis</i>

only available host, the vector acquiring the infection chiefly from the peripheral blood, which contains wandering macrophages and monocytes harbouring the parasite. In the Sudan man would also appear to be the most important source of infection of the sandfly, but, in addition to the blood, the vector may acquire the infection from dermal lesions. As regards the areas where infantile Kala-Azar is prevalent (Mediterranean littoral, Central Asia, China and probably South America) and where dogs act as reservoir hosts of human infection, the vector probably feeds on both hosts, though dogs harbouring the parasites in the skin represent a more important source of infection than man. The course of development of *L. donovani* in the intermediate host is similar to that described above. Transmission to man is effected by the bite of an infected sandfly, when the flagellate forms are introduced into the skin.

### DIAGNOSIS

The only infallible method of diagnosis of Kala-Azar (in addition to the clinical picture) is the actual demonstration of the parasites in the patient. These are sought for in organs and tissues containing fixed and wandering cells of the reticulo-endothelial system which are the only elements harbouring the leishmanias.

Material for the parasitological diagnosis of Kala-Azar is obtained from the peripheral blood, spleen, lymph glands, bone-marrow, liver and skin. The choice of methods depends partly on the worker's personal preferences and partly on the type of the disease he is dealing with.

(1) Examination of Blood.—The frequency with which leish-

manias are encountered within macrophages circulating in the blood appears to vary in different localities. Thus, in the Indian form of Kala-Azar the parasites are always present in the blood, while in the Sudanese and Mediterranean forms of the disease blood infection is scanty. Examination of the blood as a routine method is accordingly resorted to in the first case but not in the last two.

The blood is examined in stained thin blood-films, the preparation and staining of which are described in Chapter 17. Since the parasites are most likely to be found within macrophages and monocytes, they should be sought for along the margins and ends of the film. The end of the film will be thicker and the concentration of white cells higher, if the spreading of the film is interrupted before the blood on the slide is exhausted.

(2) Spleen Puncture.—Leishmanias are invariably detected in smears made from material obtained by spleen puncture and in cultures of this material. The technique of spleen puncture and preparation of smears is fully described in Chapter 17.

Though spleen puncture is one of the most reliable diagnostic methods in Kala-Azar, the operation involves a certain amount of risk to the patient. On account of this some practitioners prefer to use one of the safer methods described below.

(3) Sternal Puncture.—As the bone-marrow reveals the leishmanias in about 90 per cent. of known positive cases and because of the safety of the operation, sternal puncture is now widely used in the diagnosis of Kala-Azar (for technique see Chapter 17).

(4) Liver Puncture.—Material obtained by puncture of the liver does not always reveal the parasites in Kala-Azar patients, therefore this method is now used only when spleen puncture or sternal puncture are impracticable (for technique see Chapter 17). At present liver puncture is practically superseded by sternal puncture, though some workers still prefer it to the latter method.

(5) Gland Puncture.—Detection of leishmanias by puncture of the lymph glands is one of the safest diagnostic methods. It is now widely used in the diagnosis of the Mediterranean and Sudanese forms of Kala-Azar, in which these glands are commonly infected, but not in the case of Indian Kala-Azar, in which the examination of these glands has not given satisfactory results. The technique of gland puncture is described in Chapter 17.

(6) Examination of Skin.—As already stated, cutaneous infection with *L. donovani*, accompanying or independent of visceral infection, may be present in a high proportion of cases of Sudanese Kala-Azar, and to a lesser extent in the Mediterranean form of this disease, whereas in India it appears as a sequel to the generalized infection. In all these cases the parasites can be detected in scrapings of dermal lesions or in small thin slices excised from the skin. The material thus obtained is smeared on a slide, stained, and examined microscopically for leishmanias (for technique see Chapter 17).

(7) Culture.—*L. donovani* can be readily detected by culture in N.N.N. or Adler's media of material obtained by any of the foregoing methods (for description and technique see Chapter 19). The only disadvantage of the culture method is the time required before the results are known, for it may take from one week to about a month before the parasites can be detected in the culture. The cultures are examined by taking up a sample with a sterile pipette, placing a drop of the fluid on a slide, applying a coverslip, and examining the fresh preparation microscopically ( $\frac{1}{2}$  in. or  $\frac{1}{4}$  in. objective). The cultural forms of *L. donovani* can be easily recognized as actively motile leptomonad flagellates (Fig. 22, c-f).

### xviii. *LEISHMANIA TROPICA* (WRIGHT, 1903)

Synonym : *L. brasiliensis* Vianna, 1911.

Relation to Disease.—*Leishmania tropica* is the cause of cutaneous leishmaniasis (Oriental Sore, etc.) and mucocutaneous leishmaniasis (Espundia), diseases characterized by a local invasion by the parasites of the reticulo-endothelial cells of the skin, in some cases extending to the mucous membranes of the naso-pharynx, and manifested by the presence of sores and ulcers.

Geographical Distribution.—Like Kala-Azar, cutaneous leishmaniasis is a disease of warm countries. In the Old World it occurs in discontinuous foci over a wide area: in Southern China, in North-East India, Central Asia and the Caucasus; in the Middle East and the Arabian Peninsula; in the Mediterranean basin it is endemic all along the coasts of North Africa and Southern Europe. In the New World the disease occurs in Mexico and in most of the countries of South America.

In most cases the distribution of Oriental Sore in the Old World

is distinct from that of Kala-Azar. In some regions, e.g. the Arabian Peninsula, Kala-Azar is absent altogether and Oriental Sore occupies an independent area. In other localities the areas of distribution of the two diseases—though distinct—adjoin each other, as in India and North Africa. On the other hand, in Soviet Central Asia the distributions of visceral and cutaneous leishmaniasis overlap or coincide.

### HOST-PARASITE RELATIONSHIP

**Types of Disease.**—Like Kala-Azar, cutaneous leishmaniasis is a disease affecting man and dogs; in addition to these hosts gerbils and other wild rodents are sometimes naturally infected with *L. tropica*. There are several forms of cutaneous leishmaniasis differing in clinical manifestations. However in the Old World they have not been clearly differentiated, except in Central Asia, where Russian workers recognize the following two types of Oriental Sore, which are distinguishable on clinical, immunological and epidemiological grounds: (1) the “dry” type, which is an urban disease characterized by a chronic course and unbroken sores persisting for months before ulceration takes place, with numerous parasites in the lesions: and (2) the “moist” type, which is a rural disease characterized by an acute course and lesions ulcerating in about a week or two, with scanty parasites.

Though some forms of cutaneous leishmaniasis encountered in countries outside Central Asia are comparable to the two types just described, further investigations are required before the variants of this disease can be accurately classified.

In the New World the disease, which is usually of the same type as Oriental Sore of the Old World, may develop into a form known as mucocutaneous leishmaniasis or Espundia, with an extension of ulceration to the mucous membranes of the nose and mouth. As regards the ætiological basis for the differentiation of the clinical variants of cutaneous leishmaniasis, some authors believe that the environmental conditions of the host determine the type of disease produced, while others think that the type of infection depends upon the strain of parasite involved, different strains of *L. tropica* varying in virulence and in the degree of adaptation to the human host. The causative organism of Espundia, commonly known as *L. brasiliensis*, is probably a race of *L. tropica*.

**Course of Infection.**—As in visceral leishmaniasis, the initial lesion of Oriental Sore appears at the site of the bite by an infected sandfly, in the form of a papule, but while in Kala-Azar this lesion disappears and the infection becomes generalized, in cutaneous leishmaniasis it remains localized in the skin, where it extends in breadth and gives rise to a sore which later ulcerates. The parasites are enclosed within the macrophages infiltrating the dermal layer of the skin. As the ulcer develops and suppuration sets in the parasites become localized in reticulo-endothelial cells of the margins and the base of the lesion, and extend into the adjacent lymph nodes and into the lymphatics leading from them. The sores usually occur singly or in small numbers but occasionally they may be very numerous. Multiple lesions may be due to direct transfer to a new site, e.g. by scratching, or they may arise as the result of many bites by the vector. However, the origin of lesions widely scattered all over the surface of the body is not clear. The infection is typically restricted to the skin in the Old World and in the majority of cases in the New World but in 10–20 per cent. of cases in South America—chiefly in the tropical forest areas—the lesions involve the mucous membranes of the nose, mouth and pharynx, giving rise to the mucocutaneous form of the disease (Espundia). *L. tropica* has not been found in the peripheral blood.

The incubation period of cutaneous leishmaniasis varies from one week to several months.

**Immunity and Vaccination.**—In some cases of cutaneous leishmaniasis the infection may be inapparent, pointing to the existence of some degree of natural immunity, while the development of acquired immunity in this disease is a well-established fact. A single infection with Oriental Sore, terminating in spontaneous recovery, produces a stable immunity which affords complete protection against reinfection. This fact is well known in the East, where the natives vaccinate themselves on the arm with material from a sore, with a view to protecting themselves from disfigurement resulting from a natural infection on the face. However, there is experimental evidence that complete immunity is acquired only if the lesion is allowed to run its full course. The results of reinoculation of the parasite in the course of a pre-existing infection depend upon the phase already reached by it. In the presence of an early sore the development of the new lesion proceeds normally,

as in primary infections, but in later stages of the disease the development of the secondary lesion is retarded and the symptoms of the secondary infection (superinfection) will be the less pronounced the nearer the original one approaches to recovery. In the light of these facts it is evident that preventive vaccination of Oriental Sore can only be successful if it is timed to bring about the termination of the disease before the vaccinated person is exposed to the risk of natural infection.

### Infection in Lower Mammals

**Natural Infection.**—Dogs are found naturally infected with *L. tropica* both in the Old and in the New World. The disease in these animals is manifested by skin lesions of the same type as those developing in man. Some observers have recorded also a visceral infection in cases of canine cutaneous leishmaniasis but this is denied by others, who believe that such cases represent canine Kala-Azar, in which, as already pointed out, cutaneous and visceral infections co-exist. This question obviously stands in need of further investigation. Though the distribution of canine and human cutaneous leishmaniasis usually coincides, the rôle of dogs as reservoir hosts of the infection has not been conclusively proved. However, the identity of the parasite causing the disease in man and dogs has been demonstrated experimentally by cross-infection of these hosts.

In Central Asia various desert rodents, especially the gerbil *Rhombomys opimus*, are naturally infected with *L. tropica* which produces in them cutaneous sores on the ears. Natural infections in gerbils occur not only near the endemic foci of the human disease but also in the open desert, far from human habitations. The disease is transmitted from gerbil to gerbil by sandflies (*Phlebotomus caucasicus*) living in the burrows of these rodents. It was also demonstrated, by experimental transmission of the infection from these rodents to man and *vice versa*, that the parasites of the gerbil and man were identical, the disease produced in man being of the "moist" type. It would thus appear that in the desert areas of Central Asia Oriental Sore is a zoonosis, i.e. essentially a disease of lower animals which is communicable to man. The wild rodents (especially gerbils) serve as reservoir hosts of cutaneous leishmaniasis, which is transmitted to man by sandflies breeding in the burrows of these rodents.



The foregoing data concern the epidemiology of the "moist" type of sore, which is prevalent in rural settlements merging with the desert. As regards the "dry" type of sore in the urban form of Oriental Sore, practically nothing is known regarding its reservoir host, though domestic rodents and dogs have been suspected. The question regarding possible reservoir hosts of cutaneous leishmaniasis outside Central Asia has not been properly investigated. However, since some North African wild rodents, e.g. gerbils and ground squirrels, have proved to be susceptible to experimental infection with *L. tropica*, it is possible that also in other endemic regions burrowing rodents might serve as reservoir hosts of the human disease.

**Experimental Infection.**—In addition to the dog and some of the wild rodents mentioned above, a number of other mammals are susceptible to infection with *L. tropica*, e.g. cats, monkeys, hamsters, rats and mice. In some of these animals, e.g. monkeys, the infection is limited to the skin, while in mice the infection is sometimes purely cutaneous and sometimes involves also the internal organs.

### Transmission

As in Kala-Azar, cutaneous leishmaniasis is transmitted by sandflies (genus *Phlebotomus*). The chief vectors in different countries are shown in Table 8.

Infection of the sandfly takes place when it feeds on a cutaneous

TABLE 8  
CHIEF VECTORS OF CUTANEOUS  
LEISHMANIASIS

COUNTRIES	SPECIES OF <i>Phlebotomus</i>
Central Asia . . .	<i>P. papatasi</i> , <i>P. caucasicus</i>
India . . .	<i>P. sergenti</i>
Iraq . . .	
Syria . . .	
Palestine . . .	<i>P. papatasi</i>
Mediterranean area .	<i>P. sergenti</i> , <i>P. papatasi</i>
South America . . .	<i>P. intermedius</i>

sore, the development of the parasite in the intermediate host being similar to that described above. Transmission is effected by inoculation of the leptomonad flagellates by the bite of an infected sandfly.

### DIAGNOSIS

The diagnosis of cutaneous leishmaniasis is based entirely on the detection of the parasites in the lesions, material from which is obtained by puncture and examined in stained smears, as described in Chapter 17. The parasites are found at the sides and base of the lesions but not in the pus taken from the middle of an ulcer. They can also be cultivated from material obtained by puncture of a sore but not from the blood.

## CHAPTER 11

### THE TRYPANOSOMES

#### GENUS *TRYPANOSOMA* GRUBY, 1843

*Hæmoflagellates* of the genus *Trypanosoma*, or trypanosomes, occur in the blood and some tissues of various vertebrate animals, being common in fishes, amphibia, reptiles, birds and mammals. As in the case of leishmanias, the life-cycle of trypanosomes involves an alternation of hosts. One of these, a vertebrate, is the final host, while various blood-sucking invertebrates, especially insects, represent the intermediate hosts, or vectors, which transmit the infection to new vertebrate hosts.

Trypanosomes may be divided into *pathogenic* and *non-pathogenic* forms, according to their effect upon the vertebrate host. In animals harbouring non-pathogenic trypanosomes the infection produces no symptoms of a diseased condition, the host remaining a healthy carrier, e.g. *T. lewisi* in rats. The pathogenic trypanosomes cause definite symptoms of disease, known as *TRYPANOSOMIASIS*, which frequently terminates fatally, as in Sleeping Sickness caused by *T. gambiense* and *T. rhodesiense*, and in Chagas' disease caused by *T. cruzi*, both of which are human trypanosomiasis; as well as in a number of trypanosomiasis of domestic animals. However, the division of trypanosomes into pathogenic and non-pathogenic forms, which is convenient for practical purposes in medical and veterinary practice, does not reflect the true host-parasite relationships in trypanosomiasis, since pathogenicity is not a constant and necessary property of even the most virulent trypanosomes, for, in addition to the host to which they are pathogenic, most of the pathogenic trypanosomes may be parasitic in some other animals to which they are perfectly harmless. Such animals are merely carriers of the infection and may serve as reservoir hosts from which the infection spreads to more susceptible hosts.

As a rule, each species of mammalian trypanosome—with which we are chiefly concerned here—is restricted to a definite species or related group of vertebrate hosts. This is especially true of non-pathogenic forms which are not infective to any animals but their proper host. On the other hand, the majority of pathogenic

trypanosomes are also infective to a number of animals which are not related to their own host and do not harbour these trypanosomes under natural conditions. The virulence of trypanosomes for such mammals is used for their study under laboratory conditions and for diagnostic purposes.

### MORPHOLOGY AND LIFE-CYCLE

The developmental stages through which trypanosomes pass in the course of their life-cycle in the vertebrate and invertebrate hosts comprise the trypanosome, crithidial and leishmanial forms, and occasionally also the leptomonad form (Fig. 21).

#### The Parasite in the Mammalian Host

The most important stage of development in the genus *Trypanosoma* is the trypanosome form occurring in the blood of the vertebrate host and sometimes described as the "blood-form." The diagnosis of species is based mainly on differences in the structure of the trypanosome stage, the general morphology of which is shown in Fig. 19. Various species of *Trypanosoma* may differ in the size and shape of the body, in the position of nucleus and kinetoplast, and in the degree of development of the undulating membrane and flagellum. A well-developed undulating membrane is markedly convoluted and conspicuous, whereas an undeveloped membrane is more or less flat and inconspicuous. As regards the flagellum, it may or may not have a free portion. In the former case the trypanosome is said to have a "free" flagellum, whereas in the latter case it is said to be without a free flagellum. The movements of trypanosomes, effected by means of the flagellum with the help of the undulating membrane, are also characteristic in different species.

**Reproduction.**—Trypanosomes typically multiply by binary fission, though in some species (e.g. *T. lewisi*) division of the cytoplasm is retarded, assuming the character of multiple fission. Reproduction of different species of these parasites in the vertebrate host may be continuous or discontinuous, and may differ as regards the stage in which it takes place (see Table 9). The process of division in trypanosomes has been described in Chapter 9 and is depicted in Fig. 20.

**Cultivation.**—The ease with which trypanosomes can be cultivated

that under certain conditions they may set up a transient infection in the human host.

In view of these facts it is proposed to give a brief description of some of the most important mammalian trypanosomes as well as of a trypanosome occurring in African crocodiles, since its intermediate host is also one of the vectors of human trypanosomiasis.

The identification of the species of mammalian trypanosomes is based primarily on the morphological characters of the forms occurring in the blood of the vertebrate host and on the stages and course of their development in the insect-host, while the biological features, such as method of transmission, pathogenicity and host-specificity (or host-restriction), provide supplementary criteria for their differentiation.

The classification of the most important mammalian trypanosomes is given in Table 9. The structure of the blood-forms of these trypanosomes is shown in Plate II, while the appearance and course of development of the tsetse-borne trypanosomes in the vector are illustrated in Fig. 27 and described in Table 11.

### TRYPANOSOMES OF THE *BRUCEI-EVANSI* GROUP

Trypanosomes of the *brucei-evansi* group are characterized by polymorphism, which may be constantly present or only reveal itself sporadically. They have a small kinetoplast, which is typically subterminal, and a well developed, or conspicuous undulating membrane. This group is subdivided into (1) the *brucei* subgroup and (2) the *evansi* subgroup.

#### 1. *Brucei* Subgroup

The *brucei* subgroup comprises three species: *Trypanosoma gambiense*, *T. rhodesiense* and *T. brucei*. The first two are human parasites, causing Sleeping Sickness, while the latter is restricted to lower mammals.

The structure of these trypanosomes, which are morphologically indistinguishable, is described below.

xix. *TRYPANOSOMA GAMBIENSE* DUTTON, 1902

xx. *T. RHODESIENSE* STEPHENS & FANTHAM, 1910

Relation to Disease.—The two trypanosomes named above cause a disease, known as Sleeping Sickness, or African human trypano-

see Plate II)

D stages in the course of their

CHARACTERS

Morphology in Vertebrate —  
Kinetoplast  
Posterior end of body  
Division (stage)

Biology —  
Multiplication in vertebrate

Site of development of metacyclic trypanosomes in intermediate host

Transmission

Host-parasite relations

lands (anterior station) (except 11-13)

GROUPS

IV. BRUCEI-EVANSI

Group characters :—

Free flagellum  
Kinetoplast

Present or absent

Small, typically subterminal (absent in 12)

Specific characters —

Undulating membrane conspicuous

Polymorphic forms

SUBGROUPS :—

CEI

ii. EVANSI

ism  
ant

Polymorphism inconstant

ns with  
um and  
ns with-  
gellum  
resent

Slender forms with free flagellum  
always present, stumpy forms with-  
out free flagellum rare or sporadic

Intermediate host absent

Transmission —

velop-  
dgut +  
lands

Mechanical  
Kinetoplast —

Contact

el  
esense  
biense

Present

Absent

11. *T. evansi*

12. *T. equinum*

13. *T. equiperdum*



somiasis. The initial stages of the infection are characterized by invasion of the blood stream (parasitæmia) and lymph glands (polyadenitis) by the parasites, while in the later stages the central nervous system is involved, the parasites first penetrating into the cerebrospinal fluid and eventually extending to the brain and giving rise to symptoms of meningo-encephalitis. The course of the disease is chronic in infections with *Trypanosoma gambiense* and acute in infections with *T. rhodesiense*, usually terminating fatally in untreated cases.

**Geographical Distribution.**—Sleeping Sickness occurs exclusively in tropical Africa, its distribution covering a wide belt, lying roughly between 15° North and 15° South of the Equator. The areas of distribution of the chronic and acute forms of the disease, due to *T. gambiense* and *T. rhodesiense* respectively, are in most cases distinct, though cases of the one type may occur in localities where the other is prevalent. The endemic areas of Sleeping Sickness include the following territories: French West Africa, Portuguese West Africa, Spanish Guinea, Liberia, British West Africa (Gambia, Gold Coast, Sierra Leone and Nigeria), the Cameroons, French Equatorial Africa, Belgian Congo, Anglo-Egyptian Sudan (Southern districts of Equatoria Province), in all of which the *gambiense* disease is prevalent; British East Africa, where the *gambiense* disease occurs in Uganda and Kenya, and the *rhodesiense* disease in Tanganyika Territory and Nyasaland; Northern and Southern Rhodesia, Bechuanaland (S. Africa) and Portuguese East Africa, where the disease is of the *rhodesiense* type; it is also suspected that human trypanosomiasis may occur in parts of Abyssinia.

The distribution of Sleeping Sickness corresponds to that of the species of tsetse-flies (genus *Glossina*) concerned in the transmission of the two forms of the disease.

### MORPHOLOGY AND LIFE-CYCLE

The trypanosomes of the *brucei* subgroup (see Table 9) comprising, on the one hand, the two parasites causing human trypanosomiasis, *T. gambiense* and *T. rhodesiense*, and on the other hand, the parasite causing animal trypanosomiasis, *T. brucei*, are morphologically indistinguishable both in the mammalian and in the insect hosts, their differentiation being based entirely on physiological and epidemiological grounds. From the zoological point of view



these trypanosomes can, therefore, be regarded as three biological races belonging to one and the same species, but since they differ markedly as regards host-restriction, the type of disease produced by them, and other biological features, it is convenient for practical purposes to refer to them under separate specific names. The account which follows is restricted to the two species of human trypanosomes, while *T. brucei* and its relation to *T. gambiense* and *T. rhodesiense* will be dealt with later.

### The Parasite in the Mammalian Host

In the blood of man and other mammalian hosts *T. gambiense* and *T. rhodesiense* (Pl. II, e-h; Fig. 25, a-d) are characterized by marked polymorphism, comprising forms which differ in the shape and size of the body, presence or absence of a free flagellum, and other structural details. In all these forms the undulating membrane is conspicuous and well developed, the nucleus is more or less centrally situated, and the kinetoplast is small, occupying a sub-terminal position in the posterior end of the body. There is considerable variation in the length of these trypanosomes which has a range from  $12\mu$  to  $42\mu$ , including the flagellum.

The polymorphism of the species in question is manifested by the presence of three main types of blood-forms: slender, stumpy and intermediate. The *slender* forms (Pl. II, e; Fig. 25, a) are thin and long, measuring on the average about  $29\mu$  in length but sometimes up to  $42\mu$ ; the posterior end of the body is usually drawn out, tapering off almost to a point, with the kinetoplast situated at some distance (up to  $4\mu$ ) from the posterior extremity. These forms have a long free flagellum. The *stumpy* forms (Pl. II, g; Fig. 25, c) are stout and relatively short, measuring from  $12\mu$  to  $26\mu$  in length, and on the average about  $18\mu$ ; the posterior end of the body is broad and obtusely rounded, the kinetoplast being almost terminal in position; there is typically no free flagellum but in some individuals a short one may be present. The third type is represented by *intermediate* forms (Pl. II, f; Fig. 25, b) which measure on the average about  $23\mu$  in length, have a body of medium thickness with a blunt posterior end near which the kinetoplast is situated; a free flagellum of medium length is always present.

The proportion of trypanosomes belonging to any of these three types, which may be present in an infection, is subject to wide.

fluctuation but as a rule the slender forms are predominant. Though the slender and stumpy forms can be easily distinguished, they only represent the extremes which are connected by transitional or intermediate forms. There is also an overlap in the length of the stumpy forms ( $12-26\mu$ ), on the one hand, and the two thinner forms ( $17-42\mu$ ), on the other. The trypanosomes found in the primary lesion (see below) and in the initial infection of the blood stream belong to the slender type and are thus monomorphic, but after a few days stumpy forms begin to appear in increasing numbers and the trypanosomes become polymorphic.

In addition to the three forms just described, there occurs in these trypanosomes another type which is characterized by the displacement of the nucleus into the posterior end of the body, near or even beyond the kinetoplast. Such trypanosomes, known as "*posterior-nuclear*" forms (Pl. II, h; Fig. 25, d), usually make their appearance in the blood of experimentally infected laboratory animals. They are particularly common in *T. rhodesiense*, while in *T. gambiense* their occurrence is more rare. "*Posterior-nuclear*" forms have also been encountered in small numbers in human infections with *T. rhodesiense*. The exact relationship between the three forms and the significance of this polymorphism has not been clearly elucidated.

The polymorphism characteristic of the human trypanosomes is lost after they have been maintained for long periods of time by direct passages through laboratory animals, when they become *monomorphic*, only the slender forms being usually present in the blood. On account of this peculiarity, strains kept in laboratories outside Africa do not convey a true picture of the morphology of *T. gambiense* and *T. rhodesiense*, unless they have been recently isolated from their natural host.

In the earlier stages of the infection *T. gambiense* and *T. rhodesiense* inhabit the blood stream and lymph channels (especially in the lymph glands), later invading the cerebrospinal fluid and brain, where they are lodged in the intercellular spaces. They are thus confined to the body fluids and do not penetrate into the cellular elements. When observed in a fresh preparation of the blood, the trypanosomes are seen to be actively motile, the slender forms sometimes rapidly traversing the field, while the movements of the stumpy forms are more sluggish and restricted to a small area.

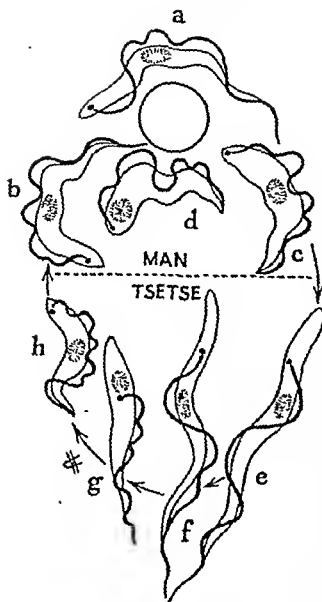


FIG. 25.—LIFE-CYCLE OF *Trypanosoma gambiense* AND *T. rhodesiense* ( $\times 2,000$ ).  
(After Hoare, from Broom, 1942.)

a-d. Trypanosomes in human blood: a. Slender form; b. Intermediate form; c. Stumpy form; d. Form with posterior nucleus. e-h. Stages in tsetse-fly: trypanosome forms (e) in stomach and (f) in proventriculus; g. Crithidial form and (h) metacyclic trypanosome in salivary glands. Erythrocyte drawn to scale.

The food of these trypanosomes is absorbed through the surface of the body from the surrounding fluid, nutrition being chiefly at the expense of blood sugar (glucose).

**Reproduction.**—*T. gambiense* and *T. rhodesiense* multiply by binary longitudinal fission in the trypanosome stage, as already described (see Chapter 9 and Fig. 20, a-e). Multiplication, which takes place in the blood, is continuous, therefore the examination of a blood-film containing trypanosomes usually reveals dividing individuals throughout the period of blood infection. In the great majority of cases multiplication is restricted to the slender forms, which are thought to represent the reproductive stage of the trypanosome, but occasionally stumpy forms also undergo division (Fig. 20, f, g).

**Cultivation.**—Like other pathogenic trypanosomes, the African human trypanosomes do not readily grow in artificial media. As already stated, the development of the hæmoflagellates (leishmanias

and trypanosomes) in culture corresponds to their development in the insect-vector, both as regards the stages through which the flagellates pass and the temperature at which they grow. In the case of *T. gambiense* and *T. rhodesiense* this parallelism goes further, their successful growth in culture being also correlated with their transmissibility by the vector. Thus, only strains which succeed in establishing an infection and completing their development in the tsetse-fly are capable of growing in culture, while those which have lost their transmissibility are not culturable. On account of this, the cultural method has only a limited application in the diagnosis of Sleeping Sickness. The best results are obtained by cultivation in Razgha's medium at 23–28° C., as described in Chapter 19. In culture the blood-trypanosomes are transformed into long trypanosomes, corresponding to the stages found in the midgut of the tsetse-fly (Fig. 25, e), but they do not give rise to the crithidial forms and metacyclic trypanosomes found in the salivary glands of the vector.

The blood-trypanosomes can also be kept alive *in vitro*, without a change in morphology, for 24 hours at 37° C. in Yorke's medium (see Chapter 19).

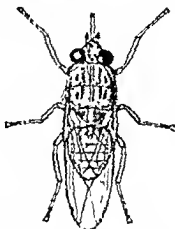


FIG. 26. — TSETSE-FLY (*Glossina*): VECTOR OF SLEEPING SICKNESS. (From Smart, 1943 *Handbook for Identification of Insects of Medical Importance*.)

### The Parasite in the Insect-Host

The intermediate host, or vector, which transmits Sleeping Sickness is the tsetse-fly, a name by which species of Diptera belonging to the genus *Glossina* are known. Though the two trypanosomes responsible for the disease are capable of developing in various species of *Glossina* (Fig. 26), under natural conditions *T. gambiense* is usually transmitted by tsetse-flies of the *palpalis* group, *G. palpalis* and *G. tachinoides*, while the chief vectors of *T. rhodesiense* are *G. morsitans*, *G. swynnertoni* and *G. pallidipes*, belonging to the *morsitans* group. Since both females and males feed on blood, flies of both sexes serve as vectors.

In the tsetse-fly the trypanosomes undergo a complicated cycle of development which takes place in the midgut and salivary glands. In order to understand the course of development of the parasite in the fly it is necessary to give a short description of its alimentary tract and accessory parts (Figs. 27 and 43).

The tsetse-fly pierces the skin of the mammalian host and ingests its blood by means of its mouth-parts, or proboscis, which consists essentially of two elongated gutter-like chitinous pieces, the labium and labrum-epipharynx (Fig. 27, l, le), the two fitting together to form a tube representing the food-canal. The proboscis encloses a long slender tube, the hypopharynx (Fig. 27, h), the anterior end of which is open, while the posterior end leads to the salivary duct. The latter emerges into the body cavity, where it divides into two ducts leading to two long tubular and convoluted salivary glands (Figs. 27, s ; 43, c) which lie in the abdominal region. The food-canal leads to the pharynx and œsophagus (Fig. 27, ph, oe) which opens into the midgut (Figs. 27 ; 43, g). From the distal end of the œsophagus a duct leading to the crop is given off (Figs. 27, c ; 43, e, f).

The anterior end of the midgut forms a piriform dilatation known as the proventriculus (Figs. 27, pv ; 43, d), while the rest of the midgut, or the stomach, is coiled up in the abdominal cavity (Fig. 43, g). The midgut is separated from the hindgut (Figs. 27 ; 43, h, i) by a sphincter, in the neighbourhood of which the two common ducts of the four Malpighian tubes open (Figs. 27, m ; 43, k). The hindgut ends in a bulbar rectum (Figs. 27, r ; 43, i) which terminates in the anal aperture.

Within the midgut, starting from the proventriculus and extending as far as the hindgut, there is a so-called *peritrophic membrane* (Fig. 27, pm), which is a thin chitinous sheath concentric with the alimentary canal. The peritrophic membrane arises in the form of a circle of droplets secreted from glandular cells situated in the wall of the proventriculus near its junction with the œsophagus. In this region there is also an annular fold which is closely applied to the anterior wall of the proventriculus. As the secretion passes between the wall and the fold it is compressed and condensed, emerging into the lumen of the midgut in the form of a thin cylindrical sleeve. Thus the anterior end of the peritrophic membrane is attached and its lumen is continuous with the œsophagus, while

posteriorly it is freely suspended, its terminal part being open at the distal end of the midgut. When blood ingested by the tsetse-fly passes into the midgut, it fills the lumen of the peritrophic membrane and is enclosed by the latter throughout the length of the midgut during the whole period of digestion. Since the trypanosomes ingested with the blood of the host are unable to pass through the peritrophic membrane, its presence has an important effect on the course of their development in the fly.

When *T. gambiense* or *T. rhodesiense* is taken up by the tsetse-fly with the blood of the host, the trypanosomes find themselves in the midgut, where they undergo certain morphological changes and assume the form of a new type of trypanosome, in which the undulating membrane is less pronounced than in the blood-form, the kinetoplast is situated about midway between the posterior end of the body and the nucleus, and a free flagellum is always present (Figs. 25, e ; 27). These trypanosomes, which vary in length up to  $35\mu$ , multiply intensively for about 10 days until very large numbers of them are present in the gut. Later on there begin to appear trypanosomes of a similar structure but of more slender build, which gradually increase in numbers and concentrate in the proventricular region, on account of which they are known as the *proventricular forms* (Figs. 25, f ; 27).

Up to the fourth day the trypanosomes remain inside the lumen of the peritrophic membrane ; here they gradually migrate backwards until they reach the open end of the peritrophic membrane, from which they escape and penetrate into the space between the peritrophic membrane and the wall of the gut. Here they begin to migrate forwards until they reach the proventriculus, in which they find themselves in a *cul-de-sac* (see Fig. 27, pv). The ultimate destination of the trypanosomes is the salivary glands, into which they can only penetrate *via* the œsophagus. However, since the proventricular forms are incapable of perforating the chitinous wall of the peritrophic membrane they penetrate through the least resistant part of it, namely at the points adjoining the annular fold, where it is secreted and is still fluid. Having thus found their way into the inner lumen of the proventriculus the trypanosomes proceed to migrate forwards, first into the œsophagus and pharynx and then into the proboscis, where they enter the hypopharynx through its open anterior end. Once in the hypopharynx, the trypanosomes

again turn back, into the salivary duct, finally reaching the salivary glands, where they complete their development.

In the salivary glands the proventricular trypanosomes attach themselves to the walls by means of the flagellum or lie freely in the lumen, and are transformed into *crithidial forms* (Figs. 25, g ; 27). These multiply and undergo further transformation, giving rise to a new type of trypanosome, known as *metacyclic trypanosomes* (Figs. 25, h ; 27 \*), which are small forms, without or with a short flagellum, somewhat similar to the stumpy blood-forms. This completes the development of the parasite in the tsetse-fly, the metacyclic trypanosomes constituting the *infective forms*.

**Cyclical Transmission.**—The entire cycle in the fly, from the infecting meal to the production of metacyclic trypanosomes, occupies from 15 to 35 days. Until the metacyclic forms are produced the tsetse-fly is not infective, the gut forms of the trypanosome being incapable of infecting the mammalian host. Infection takes place when the tsetse-fly bites a new host and the metacyclic trypanosomes are injected with the saliva into the wound caused by the bite. The number of metacyclic trypanosomes introduced with each bite—as calculated by inducing the fly to probe on a slide (see Chapter 20)—varies considerably and may reach several thousand.

There is reason to believe that a tsetse-fly, once infected, is capable of transmitting the trypanosomes for the rest of its life, i.e. several months. However, the infectivity of *T. gambiense* and *T. rhodesiense* for tsetse-flies is relatively low, for even under experimental conditions, when all the flies are known to have ingested trypanosomes, it is rare for more than 10 per cent. to become infected. However, in "wild" flies caught in endemic regions of Sleeping Sickness the proportion containing trypanosomes in the salivary glands (i.e. belonging to one of the species of the *brucei* subgroup) is considerably lower, usually not exceeding 1 per cent. The majority of tsetse-flies thus appear to be naturally resistant to infection with the trypanosomes in question.

The trypanosomes themselves also vary in their power to infect tsetse-flies. It is known that strains of *T. gambiense* and *T. rhodesiense*, which formerly completed their cycle of development in the tsetse-fly and were transmissible to new mammalian hosts, gradually lose their power to develop in the vector. There are

different degrees of this disability: in some cases the trypanosomes start developing in the gut but are unable to invade the salivary glands and complete their life-cycle; in others there is complete failure to develop even in the gut. Such strains of trypanosomes, which are obviously unable to transmit the infection, are accordingly known as *non-transmissible* strains.

In general, the ability of a strain to complete its development in the intermediate host, or its transmissibility, is influenced, on the one hand, by the temperature to which the flies have been exposed either in the pupal or in the adult stages, and, on the other hand, by the host from which the flies have acquired the infection. The infection rate is greater and the duration of the development of the trypanosomes in the tsetse-fly is shorter at higher temperatures. The difference in the percentage of wild flies found infected in the cool and hot seasons may thus be due to variations in the temperature of the environment.

The infectivity of the trypanosomes for *Glossina* is highest in the early stages of Sleeping Sickness but gradually declines as the disease progresses and may be lost by strains from human cases of long-standing chronic infections or after prolonged maintenance in other mammals. The impairment of the power of the trypanosome to establish itself in the tsetse-fly is usually preceded by a period during which there has been no contact between the parasite and its intermediate host—in other words, cyclical transmission through the insect had for some time been eliminated from the life-cycle of the flagellates. As stated above, loss of transmissibility by the trypanosome is also correlated with its inability to develop in culture.

**Mechanical Transmission.**—In addition to cyclical transmission, following the completion of their development in the natural vectors, the African human trypanosomes may be transmitted mechanically both by tsetse-flies and by other insects. In the case of blood-sucking insects (e.g. tsetse-flies, horse-flies, stable-flies, mosquitos) the trypanosomes taken up with the blood of the mammalian host may survive for a short time in the proboscis and when the insect bites a new host the trypanosomes are transferred to it with the saliva. In such cases the trypanosomes do not undergo any development in the insect but retain the structure characteristic of the blood-forms, while the insect acts merely as a syringe, as in experi-



mental inoculations of laboratory animals. Some epidemic outbreaks of Sleeping Sickness have been attributed to mechanical transmission by *Glossina*.

It is thought that, under certain conditions, non-blood-sucking flies might also transmit trypanosomes mechanically. Thus, *Musca spectanda*, which occurs in some parts of Africa, habitually feeds on the blood of mammals (including man) exuding from scratches, wounds, sores and abrasions on the skin, which are so common in the tropics. In this way they may ingest the trypanosomes, which pass through their gut and are discharged alive with the faeces on to open wounds or sores of another mammal, which thus becomes infected.

### HOST-PARASITE RELATIONSHIP

**Types of Disease.**—Sleeping Sickness or African human trypanosomiasis occurs in two main forms, the Gambian and Rhodesian, which are attributed to distinct species of trypanosomes (*T. gambiense* and *T. rhodesiense* respectively) and differ in the course

TABLE 10  
DIFFERENTIAL CHARACTERS OF SLEEPING SICKNESS

Types of Disease		GAMBIAN	RHODESIAN
Causative Organisms		<i>T. gambiense</i>	<i>T. rhodesiense</i>
Disease in Man		Chronic, lasting up to several years; pronounced nervous symptoms	Acute, lasting 3-9 months; toxic symptoms predominating
Reaction to Arsenicals (Tryparsamide)		Sensitive	Resistant
Vectors ( <i>Glossina</i> )		<i>palpalis</i> group	<i>morsitans</i> group
Behaviour in laboratory animals	Virulence	Low	High
	Posterior-nuclear trypanosomes	Usually rare	Common

and duration of the disease, geographical distribution, vectors, reaction to treatment with arsenical drugs and behaviour in laboratory animals. The essential characters of the two types of the disease are given in Table 10.

The distinguishing features of the Gambian and Rhodesian diseases shown in Table 10 are characteristic of typical cases, but actually the clinical manifestations attributed to *T. gambiense* and *T. rhodesiense* are not so clearly demarcated as is usually assumed, for cases showing symptoms of one type of Sleeping Sickness may occur in the area of distribution of the other type. Thus in West Africa and in the Belgian Congo, where the Gambian form is prevalent, in the majority of cases the disease runs a chronic course lasting several years, sometimes even without showing any clinical symptoms whatever, but in a minority of cases the disease is acute, resistant to treatment with tryparsamide, and the patients die within a few months of the onset of the disease. Such cases of the Gambian disease, occurring chiefly in epidemic outbreaks on first introduction of the infection into a locality, are indistinguishable from the Rhodesian disease. On the other hand, in Tanganyika Territory, where the Rhodesian form is prevalent and in the majority of cases the disease runs an acute course lasting only a few months, the disease sometimes assumes a chronic form indistinguishable from the Gambian disease.

Some of the other differential characters of the two forms of Sleeping Sickness are also of relative value. Thus, the virulence of *T. gambiense* for laboratory animals can be increased by direct passages until it is similar to that of *T. rhodesiense*. As regards the vectors, both human trypanosomes are capable of developing in the same species of *Glossina* under experimental conditions, while under natural conditions strains indistinguishable from *T. rhodesiense* have been isolated in areas where the vector is *G. palpalis*. The resistance of *T. rhodesiense* to the action of arsenicals is likewise not an absolute differential character, for both in East and in West Africa there have been recorded cases of infection with strains of *T. gambiense* which were resistant to treatment with tryparsamide.

There is thus no hard and fast line separating the diseases caused by *T. gambiense* and *T. rhodesiense*. There can be no doubt that these two species are very closely related and probably identical,

*T. rhodesiense* representing merely a virulent strain or race of *T. gambiense*. There are some indications that these two trypanosomes are convertible into each other, thus after cyclical passages by tsetse-flies through sheep for several years *T. rhodesiense* may acquire the characteristics of *T. gambiense*.

The position regarding Sleeping Sickness can be summarized as follows. The disease is caused by a trypanosome of the *brucei* subgroup varying in virulence to man. The course of the disease may be (1) mild and symptomless, (2) chronic or (3) acute. In some localities the mild and chronic types of the disease predominate, and are associated with vectors of the *palpalis* group of tsetse-flies: in such cases the disease is said to be of the *Gambian* type. In other localities the acute type of the disease is more common and the vectors belong to the *morsitans* group: in such cases the disease is said to be of the *Rhodesian* type.

Course of Infection.—As already mentioned, infection with *T. gambiense* and *T. rhodesiense* is produced by inoculation of the metacyclic trypanosomes into the skin of the mammalian host through the bite of the tsetse-fly, the number of trypanosomes thus injected varying considerably in numbers. There is reason to believe that man requires a *minimum* dose of from 300 to 450 metacyclic trypanosomes for infection to be successfully established.

The first sign of infection appears at the site of the bite by an infected tsetse-fly as a local cutaneous reaction in the form of an indurated nodule, which is first as large as a pea but gradually increases in size until it becomes a swelling measuring about 3 in.  $\times$  2 in. Puncture of this primary lesion may reveal trypanosomes in the blood and serous exudate withdrawn from it some days before the blood stream is invaded. The primary lesion may be accompanied by high temperature and swelling of the adjacent lymph glands. The appearance of the nodule is the earliest diagnostic character of Sleeping Sickness, since bites by non-infected tsetse-flies do not produce this reaction.

The incubation period of Sleeping Sickness, determined by the first day when trypanosomes can be detected in the blood of the patient, varies from 7 to 16 days but is sometimes longer, the difference depending upon the virulence and number of metacyclic trypanosomes inoculated, on the one hand, and upon the resistance of the human host, on the other hand.

Both in the primary skin lesion and in the early blood infection the great majority of trypanosomes are represented by slender forms with a definite free flagellum (Pl. II, e, f). During the first period of the infection there is extensive multiplication of the trypanosomes in the blood (parasitæmia) and in the lymph glands, producing, on the one hand, fever, and on the other, enlargement of the glands, owing to local proliferation of phagocytes as a defence reaction against invasion by the parasites. During this phase of the infection the trypanosomes appear in the blood periodically and then disappear. The periodical disappearance of the trypanosomes, or crisis, is due to their destruction in the blood by trypanolytic antibodies aided by leucocytes, which dispose of the remains of the parasites but apparently do not phagocytose the trypanosomes while they are undamaged. It is thought that the mass destruction of trypanosomes occurring during the crisis releases into the circulation an endotoxin which brings about a febrile reaction on the part of the host. Though a large proportion of the parasites are thus destroyed, a certain percentage which are more resistant to the trypanocidal action of the antibodies survive and repopulate the blood stream, giving rise to a relapse strain of trypanosomes. The periodical appearance and disappearance of trypanosomes in the blood is due to the production of a series of such relapse strains, each resistant to the action of the antibodies evoked by the preceding strain. As the infection progresses the trypanosomes become scantier in the blood and may be absent for months in old-standing untreated cases, but they can usually be found in the lymph glands after they have disappeared from the blood. They persist for a longer period in the blood of patients suffering from the Rhodesian disease, in which examination of the blood nearly always reveals the parasites. The trypanosomes are more likely to be detected in the blood when there is a rise of temperature.

After a variable period of time—about one month in the case of the Rhodesian disease and several months in the Gambian form—the trypanosomes invade the cerebrospinal fluid, where they are usually found in small numbers, as a rule not exceeding 1 per 1 ml. of the fluid, more than 50 being an exception. The trypanosomes in the cerebrospinal fluid always have a free flagellum and may be more slender than the corresponding forms in the blood, while some show signs of degeneration. As the disease progresses the

may run a chronic course lasting up to several years (in the Gambian form) or an acute course lasting only several months (in the Rhodesian form), in both cases terminating fatally if untreated. Some of the pathological effects of trypanosomiasis may also be due to the impairment of the glycogenic functions of the liver, producing a state of hypoglycemia which is brought about by consumption of the blood sugar (glucose) by the trypanosomes.

There have been reported a number of instances of *congenital transmission* of Sleeping Sickness from mother to foetus, which takes place through the placenta, as the result of vascular changes in the latter, enabling the trypanosomes to penetrate into the foetal circulation.

**Immunity.**—The question of immunity in African human trypanosomiasis has not been thoroughly investigated. Recent work on volunteers has shown that there is individual variation in the susceptibility to infection with trypanosomes and that a relatively large number of metacyclic trypanosomes must be inoculated by the vector before they can establish themselves in man. This seems to indicate that there is a degree of natural resistance to invasion. There is also some evidence of acquired immunity in localities where Sleeping Sickness has been endemic for a long time. In certain of these areas the natives do not show any symptoms of disease though trypanosomes are present in their blood. Such persons represent more or less healthy carriers and the immunity acquired by them is probably of the type known as *premunity*, in which the parasites are not wholly destroyed but their numbers are kept at a low level. Apparently the development of immunity is a slow process, since an infection which has been successfully treated with drugs does not confer immunity against re-infection, as shown by the fact that volunteers can be infected repeatedly. Likewise, antelopes infected with *T. rhodesiense* and pigs infected with *T. gambiense* can be re-infected with these trypanosomes after spontaneous recovery from the infection.

As regards the mechanism of immunity, it appears to be mainly humoral, with phagocytes playing a subsidiary rôle. As shown above, the infection is characterized by a succession of crises and relapses: when the parasites in the blood reach a certain concentration most of them are destroyed by the trypanolytic action of the antibodies but a small number survive and give rise to a relapse

strain with different antigens, which in its turn is destroyed by new lysins. The various relapse strains differ from each other serologically and can be distinguished by cross-immunity tests.

The presence of strain-specific immune bodies in trypanosomiasis can be demonstrated by taking a sample of serum from an infected animal which has recovered from the infection and mixing it with trypanosomes. If the trypanosomes are homologous, i.e. belong to the strain which produced the original infection, they will be covered with blood-platelets or red blood corpuscles, if these have been added to the mixture, but if the trypanosomes belong to a different (or heterologous) strain of the same species, there will usually be no reaction. This phenomenon is known as the ADHESION TEST. The existence of trypanocidal antibodies is also evident from the fact that growth of the trypanosomes in culture is inhibited if the blood used in the preparation of the medium is taken from a patient suffering from Sleeping Sickness or from an experimentally infected animal.

As a general rule, man is not susceptible to infection with trypanosomes of other mammals (e.g. *T. brucei*). This is due to the fact that normal human serum kills them, i.e. it is trypanocidal, whereas it has no harmful effect upon *T. gambiense* and *T. rhodesiense*. However, *T. rhodesiense* is liable to lose its serum-resistance temporarily, when passaged through other animals, but recovers it when re-introduced into man.

#### Infection in Lower Mammals

In addition to man, *T. gambiense* and *T. rhodesiense* are infective to a wide range of mammals, some of which occur in the endemic areas and may be naturally infected with these trypanosomes, while others do not normally come in contact with them but are susceptible to experimental infection. As in the case of human infections, *T. rhodesiense* is more virulent to lower mammals than *T. gambiense*.

Experimental Infection.—Both African human trypanosomes produce heavy infections, with symptoms resembling those in the human host, in most monkeys, except baboons which are refractory. They are also infective to various laboratory rodents (mice, rats, guineapigs, rabbits) but whereas *T. rhodesiense* usually succeeds in establishing itself in these animals directly, they are not readily infected with *T. gambiense* on first isolation from man, but after

a strain of this trypanosome has passed through a monkey it becomes more virulent for the rodents. The inoculation of such animals, especially rats, with the blood from a suspected human case is used as an aid to the diagnosis of the infection. Other animals susceptible to infection with the human trypanosomes include dogs, cats, sheep, goats, cattle, antelopes, domestic and wild pigs, and hyrax.

The course of experimental infection in these animals varies. In rats and mice the trypanosomes in the blood multiply rapidly and unimpeded, producing a state of parasitæmia which usually kills the host in several days. In guineapigs the infection tends to assume a more chronic form, with a succession of crises and relapses similar to that observed in the human host during the period of blood infection, but ultimately terminating in the death of the animal.

In the case of some of the larger mammals there is a higher degree of mutual adaptation between the parasite and the host. Thus, both *T. gambiense* and *T. rhodesiense* can be maintained in some antelopes and in wild pigs for periods up to 2 years, without producing any symptoms of disease and terminating in spontaneous recovery of the host. Experimental infection of goats with *T. gambiense* runs a similar course. However, in other antelopes, as well as sheep and hyrax, infected with *T. rhodesiense*, the cerebrospinal fluid becomes involved and the animals succumb to the infection.

**Natural Infection.**—Natural infections with trypanosomes of the *brucei* subgroup are quite common among wild and domestic mammals living in the endemic areas of Sleeping Sickness and in tsetse-flies caught in these localities. Natural infections with these trypanosomes have been recorded from various antelopes, buffalo, elephant, warthog, as well as from dogs, cattle, sheep, goats, pigs and dogs.

In view of the absence of morphological differences between the three members of this group, *T. brucei*, *T. rhodesiense* and *T. gambiense*, their identity in natural infections can be established with certainty only by observing the course of the infection in one of the susceptible animals. The course of the infection in the blood of the natural host is usually chronic and may last for years. Experimental infection of these animals is usually fatal. The purpose of this paper is to describe the course of the infection in these animals.

some instances, in most cases the nature of the trypanosomes detected in natural infections is determined from circumstantial or indirect evidence, provided by the epidemiological data.

From the epidemiological point of view, trypanosomes found in animals from localities where the human disease is unknown most probably are *T. brucei*, while those occurring in endemic areas of the Rhodesian disease may be either *T. rhodesiense* or *T. brucei*. Since both these species are highly virulent for laboratory animals, they can only be differentiated by inoculating human volunteers. If the result is positive, the trypanosome in question is *T. rhodesiense*; if it is consistently negative, the parasite is probably *T. brucei*. In endemic areas of the Gambian disease the differential diagnosis of *T. brucei* and *T. gambiense* is based on the relative virulence of the trypanosome for laboratory rodents, which are highly susceptible to infection with the former species but may be refractory to the latter.

**Reservoir Hosts.**—The occurrence of natural infections with human trypanosomes among wild and domestic animals has an important bearing on their rôle as reservoir hosts of Sleeping Sickness. In addition to the epidemiological data noted above, significant results, throwing light on this problem, have been obtained from observations on experimental infections of various animals. It has been demonstrated that *T. rhodesiense* can be maintained by cyclical transmission through antelopes and through sheep for 10 years, in the course of which it remains infective to man. Similar observations on *T. gambiense* have shown that it can be transmitted cyclically through domestic pigs, goats and dogs for periods up to 4 years, without losing its transmissibility to man. In individual animals the infection may persist for long periods of time. Thus, in some antelopes the infection with *T. gambiense* and *T. rhodesiense* may last for about 2 years, while *T. gambiense* may persist for upwards of one year in pigs and goats. During the period of infection trypanosomes are recoverable from these animals and are transmissible by tsetse-flies for several months.

From these observations and from epidemiological considerations it is concluded that in Sleeping Sickness, in addition to man, various other mammals constitute a source of infection, the relative importance of the human and animal reservoirs differing according to the type and distribution of the disease. The Rhodesian disease,



which is usually encountered on the fringes of thinly populated bush-country abounding in big game, is transmitted by tsetse-flies from man to antelopes and from these back to man, antelopes representing important reservoir hosts even in localities which have been depopulated for some years. In the Gambian disease, which occurs in relatively well populated localities with a sparse mammalian fauna, man appears to be the essential host and main source of infection, but in view of the prolonged survival of the trypanosome in antelopes and domestic animals (especially pig, goat and dog) without loss of cyclical transmissibility to man, these animals should be regarded as potential reservoir hosts.

### DIAGNOSIS

As in other protozoal diseases, the detection of the parasites in the patient is the only reliable method of diagnosis of Sleeping Sickness, the clinical symptoms of which are not always sufficiently characteristic. The methods employed, which vary according to the stage and type of the disease, are as follows.

(1) *Examination of Primary Lesion.*—In recently infected cases it should be possible to detect the trypanosomes by puncture of the skin nodule (see Chapter 17), which represents the earliest diagnostic character of Sleeping Sickness, and to treat the patient before any clinical symptoms develop. However, this method is of no practical value, for the primary lesion usually escapes notice, chiefly because the patients are rarely seen in the initial stage of the infection.

(2) *Examination of Blood.*—In the early stages of Sleeping Sickness trypanosomes occur in the blood of the patient but their numbers are usually scanty (especially in the Gambian disease) and they tend to disappear periodically, the periods of absence increasing as the disease progresses. In general the trypanosomes can be more readily detected in the blood when there is a rise of temperature. It might therefore be necessary to examine the blood repeatedly before the trypanosomes can be found. Whereas in the Gambian disease examination of the blood may fail to reveal any trypanosomes, in the Rhodesian disease the infection can always be diagnosed by blood examination. This method is accordingly of primary importance in the diagnosis of the latter disease, but only of secondary importance in the case of the former.

The blood is examined by several methods (see Chapter 17):

(a) in fresh preparations between slide and coverslip, when the presence of trypanosomes is revealed by their movements; (b) in thick and thin blood films stained by one of the Romanovsky methods; (c) after centrifugation, when other methods of blood examination have failed to reveal the parasites.

(3) Examination of Lymph Glands.—In the case of Gambian Sleeping Sickness examination of the juice obtained by gland puncture is one of the surest methods for detecting the trypanosomes during the first stage of the disease, positive results being obtained in about 90 per cent. of cases. However, in the Rhodesian disease it is not necessary to resort to gland puncture, for the trypanosomes are more readily detected in the blood.

The material obtained by gland puncture (for technique see Chapter 17) is examined fresh between slide and coverslip. If trypanosomes are present they are revealed by their movements among masses of leucocytes.

(4) Examination of Cerebrospinal Fluid.—In advanced stages of Sleeping Sickness, when the central nervous system is involved, trypanosomes can be found in the cerebrospinal fluid. The technique of lumbar puncture, centrifugation of the fluid and method of examination of the trypanosomes are fully dealt with in Chapter 17.

(5) Inoculation of Animals.—The infection can sometimes be demonstrated by inoculation of the patient's blood into susceptible animals but this method is of uncertain value. The animals commonly used for these tests are white rats, which are inoculated intraperitoneally with 1–2 ml. of the patient's blood. In most cases *T. gambiense* fails to infect rats or the incubation period is prolonged, therefore this animal is of doubtful value in the diagnosis of the Gambian disease but monkeys (e.g. *Cercopithecus*) are usually readily infected when inoculated with large amounts of blood. On the other hand, rats are, as a rule, highly susceptible to infection with *T. rhodesiense* which may appear in their blood after several days, though occasionally this trypanosome also fails to infect rats.

(6) Culture.—Cultivation of the patient's blood in Razgha's medium (see Chapter 19) is of limited value in the diagnosis of Sleeping Sickness, in view of the fact that only strains of trypanosomes which are transmissible by the vector succeed in establishing themselves in culture.

As will be shown later, American human trypanosomiasis can be successfully diagnosed by allowing several individuals of the insect-host to feed on a suspected case and examining them after a few days for the presence of developmental stages of the trypanosome. However, in African human trypanosomiasis this method, known as XENODIAGNOSIS, has no practical value, for, owing to the extremely low infection rate of the tsetse-flies, its application would require a very large number of flies and would therefore be too laborious.

The diagnostic procedure, especially in surveys of Sleeping Sickness or when a large number of persons have to be examined, can be standardized as follows. First, a thick blood film is made; while this is staining a lymph gland is punctured and the juice examined fresh. If trypanosomes have been detected in the gland-juice the blood film is discarded, but if the results of examination of the juice are negative, the stained blood film is examined. If the results of these examinations are negative or if there are signs of involvement of the central nervous system, a lumbar puncture is made and the cerebrospinal fluid is examined for trypanosomes.

In epidemiological surveys it may be necessary to determine the incidence and nature of trypanosome infections among local species of *Glossina*. For this purpose "wild" tsetse-flies are dissected or induced to salivate on a slide (for methods see Chapter 20) and the species of trypanosomes found in the insects can be differentiated with the aid of the diagram in Fig. 27 and Table 11. The trypanosomes can also be identified when they appear in the blood of susceptible animals after these have been exposed to the bites of infected tsetse-flies.

#### xxi. *TRYPANOSOMA BRUCEI* PLIMMER & BRADFORD, 1899

Like the two trypanosomes causing Sleeping Sickness in man (*T. gambiense* and *T. rhodesiense*), *T. brucei* belongs to the *brucei-evansi* group (see "Key" in Table 9). This trypanosome is parasitic in various ungulates (antelopes, cattle, equines, etc.), causing in some of them a fatal disease known as Nagana, and is widely distributed throughout most of tropical Africa in regions where tsetse-flies occur.

Morphologically *T. brucei* is indistinguishable from *T. rhodesiense* and *T. gambiense* (Pl. II, e-h) but shows more points of similarity

with the former species, since in infections of laboratory animals it gives rise to numerous "posterior-nuclear" forms. *T. brucei* also resembles *T. rhodesiense* in the high degree of virulence for various mammals, including rodents, and, like the human trypanosome, it is transmitted by tsetse-flies of the *morsitans* group, in which its development is similar to that described for *T. gambiense* and *T. rhodesiense*.

The only feature distinguishing *T. brucei* from the last-named trypanosomes is its inability to infect man. Numerous attempts to produce infection experimentally in man have failed. *T. brucei* occurs naturally in various kinds of wild game, especially in antelopes, in which the infection runs a symptomless course. Antelopes represent the natural hosts of this trypanosome and serve as reservoirs from which the infection is spread to domestic animals.

#### RELATIONSHIP BETWEEN TRYPANOSOMES OF THE *BRUCEI* SUBGROUP

It has already been shown that the three species of trypanosomes comprising the *brucei* subgroup, *T. gambiense*, *T. rhodesiense* and *T. brucei*, are morphologically indistinguishable from each other both in the mammalian host and the insect-host, but differ in their power to use man as a host and in their virulence for other mammals. However, the difference in virulence is not apparent in antelopes, for all the three trypanosomes usually produce symptomless infections in these animals. The only method by which the species of trypanosomes responsible for these infections can be determined is by testing their ability to infect man and by their effect upon laboratory animals.

From these facts it is evident that the three trypanosomes in question are closely related zoologically and might be regarded not as distinct species but rather as biological races of one and the same species adapted to different hosts—*T. gambiense* and *T. rhodesiense* to man, and *T. brucei* to lower mammals—though both the human and the animal trypanosomes have common reservoir hosts. However, it is convenient for practical purposes to retain the separate specific names by which these trypanosomes have been known for many years.

The affinities between the trypanosomes of the *brucei* subgroup

have both a theoretical and a practical interest, for there can be no doubt that they are phylogenetically related, and there are reasons to believe that the human trypanosomes have originated from *T. brucei*. This question has an important bearing on the epidemiology of Sleeping Sickness, for if it could be proved that *T. brucei* is capable of establishing itself in man, both wild animals harbouring this trypanosome and domestic animals suffering from Nagana would have to be reckoned with as dangerous sources of human infection. However, there is considerable controversy on this matter. Apparently the chief factor which prevents *T. brucei* from gaining a footing in man is the trypanocidal action of normal human serum, to which the human trypanosomes are resistant, while *T. brucei*—in common with other animal trypanosomes—is highly susceptible. But, since human serum may lose its trypanocidal properties under certain conditions (e.g. in some diseases of the liver and in avitaminoses), some workers maintain that persons suffering from such disorders might become susceptible to infection with *T. brucei*. It has also been suggested that exceptionally virulent strains of this trypanosome might be capable of overcoming the trypanocidal action of normal human serum. According to this hypothesis, having thus established itself in man *T. brucei* would gradually adapt itself to the new host and at first behave like *T. rhodesiense*—with which it has so many features in common—giving rise to an acute disease. Subsequently the adaptation would become more perfect and the trypanosome would acquire the characteristics of *T. gambiense*, giving rise to a mild or chronic disease. Adherents of this view also believe that, after passages through lower mammals for prolonged periods, the human trypanosomes may revert to *T. brucei*.

Though on theoretical grounds this appears to be a plausible hypothesis, it cannot be accepted unreservedly for several reasons. In the first place, it is well known that in enzootic areas of *T. brucei*, where human beings are constantly exposed to the bites of infected tsetse-flies, no cases of human infection have ever been recorded. Secondly, all attempts to infect human volunteers with *T. brucei* have so far failed, though in one case a transitory infection was produced, while the few reports of accidental infection of man with this trypanosome are entirely unfounded. Finally, it has been demonstrated that *T. rhodesiense* can be transmitted cyclically

through ruminants for many years without losing its power to infect man again, in other words—without reverting to *T. brucei*. However, *T. gambiense* can be converted into a virulent strain indistinguishable from *T. brucei* by passage through very young rats.

From these conflicting data it can be concluded that *T. brucei* does not readily adapt itself to man and that its transformation into *T. rhodesiense* does not occur under normal conditions. Nevertheless it is reasonable to assume that this evolution has actually happened in the remote past and might even take place at present under exceptionally favourable conditions, the nature of which is not yet clear.

## 2. *Evansi* Subgroup

The most important species of the *evansi* subgroup (see Table 9) are *Trypanosoma evansi* and *T. equiperdum*. These trypanosomes, which are morphologically indistinguishable, are typically monomorphic, the predominant forms being identical with the slender and intermediate forms in the *brucei* subgroup (Pl. II, e, f). However, occasionally very small numbers of stumpy forms (Pl. II, g) are also seen, while in some strains of *T. evansi* the stumpy forms—including those with posteriorly displaced nuclei ("posterior-nuclear" forms)—appear in fair numbers at irregular periods of time. At such periods *T. evansi* is indistinguishable from trypanosomes of the *brucei* subgroup (*T. brucei*, *T. gambiense*, *T. rhodesiense*).

## xxii. *TRYPANOSOMA EVANSI* STEEL, 1885

*T. evansi* is parasitic in various domestic animals, especially horses, cattle and camels, in which it causes a disease generally known as Surra, which varies in severity in different localities. This trypanosome comprises strains which differ from each other in their effect upon various mammals. Thus, some strains are adapted chiefly to equines, others to bovines, and others again to camels. *T. evansi* is transmitted mechanically by various blood-sucking Diptera, especially horse-flies (*Tabanus*).

The disease caused by *T. evansi* is widely distributed in warm countries throughout the world. Its distribution in Africa is of particular interest. In this continent Surra occurs wherever its principal host, the camel, is found. It is distributed over a wide

area from the Red Sea to the Atlantic Ocean, extending in the north to the Mediterranean coast and in the south to a line roughly north of 15-16° N. Lat., which is also the northern boundary of the tsetse-fly zone.

In view of the morphological similarity of *T. evansi* and *T. brucei*, including the sporadic occurrence of marked polymorphism in the former species, there can be no doubt that they are closely related, and it is probable that *T. evansi* has originated from *T. brucei*. It is conceivable that infection with *T. brucei* was contracted by camels penetrating into the tsetse-belts, whence, returning northwards, they spread the disease among other camels. Having in the course of time adapted itself to mechanical transmission by horse-flies and lost its ability to develop in tsetse-flies, *T. brucei* acquired all the characteristics of *T. evansi*. Since the camel has for centuries been the chief transport animal in North Africa and in Asia, the disease originally contracted in tropical Africa was probably gradually spread by caravans to camels and other domestic ungulates in different parts of the Old World.

In view of the proximity of the endemic areas of camel Surra and tsetse-borne trypanosomiasis, it is possible that *T. evansi* is occasionally introduced into the tsetse-zone. The occurrence of *T. evansi* in areas of tsetse-borne trypanosomiasis should therefore be taken into consideration when dealing with trypanosomes encountered in local animals.

The only member of the *evansi* subgroup distinguishable morphologically from the others is *T. equinum* Voges, 1901. This trypanosome has the general appearance of *T. evansi* but differs from it in the permanent absence of a kinetoplast. Individuals devoid of this structure are encountered in varying proportions in all species of trypanosomes but they are particularly numerous in *T. evansi*, in which totally "akinetoplasmic" strains sometimes arise spontaneously in individual hosts and continue to breed true. Such strains can also be produced artificially by exposing the trypanosomes to the action of certain dyestuffs. It is thought that under natural conditions they arise by mutation in the trypanosomes and it is probable that *T. equinum* has originated from *T. evansi* in this manner.

*T. equinum* occurs in South America, where it causes in horses a disease like Surra, known as Mal de Caderas.

xxiii. *TRYPANOSOMA EQUIPERDUM* DOFLEIN, 1901

*T. equiperdum* (Pl. II, c, f) is morphologically indistinguishable from *T. evansi*. It occurs in horses in Southern Europe, in Asia, North and South Africa, and parts of North America, causing a peculiar disease known as Dourine. The disease runs a chronic course, lasting from 6 months to 2 years and usually terminating fatally.

In infected horses the trypanosomes are found chiefly in the serous exudate of the œdematous swellings which develop in the external genital organs of the horse and in flat swellings, or plaques, on the skin. Though present in the blood, the trypanosomes are too scanty to be detected by direct examination.

Dourine is essentially a venereal disease and is transmitted during the sexual act by contact from stallion to mare and *vice versâ*, the trypanosomes being introduced through abrasions on the external genital organs. This is the only trypanosome in which transmission is effected from mammal to mammal without the intervention of an insect vector.

TRYPANOSOMES OF THE *VIVAX* GROUP

Trypanosomes of the *vivax* group (see "Key" in Table 9) have a characteristic appearance and cannot be confused with any other pathogenic trypanosomes. They are monomorphic, i.e. represented by one structural type (Pl. II, i, k); the body is somewhat club-shaped, being typically swollen and rounded at the posterior end and tapering toward the anterior end. The undulating membrane is usually feebly developed, or inconspicuous, and there is a fairly long free flagellum. The kinetoplast is usually terminal and very large, being considerably larger than that in any other of the pathogenic trypanosomes of Africa.

These trypanosomes are infective to various wild and domestic ungulates, but most laboratory animals, except rabbits, are refractory to infection. In rabbits these trypanosomes may set up a transient infection.

Trypanosomes of the *vivax* group are transmitted by tsetse-flies, the chief vector being *Glossina palpalis*, in which the infection rate may be up to 20 per cent. The development in the intermediate host takes place in the proboscis exclusively (see Fig. 27 and Table 11).



When ingested with the blood of the mammalian host the trypanosomes are taken into the proboscis and midgut. However, they do not develop further in the midgut but degenerate and are finally digested, whereas those retained in the proboscis undergo the typical cycle of development. They are first transformed into crithidial forms which attach themselves to the walls of the proboscis in large numbers and later invade the hypopharynx, where they give rise to the infective metacyclic trypanosomes. The developmental forms in the tsetse-fly retain the large kinetoplast of the blood-forms, which—among other characters shown in Fig. 27 and Table 11—distinguishes them from the developmental stages of other species of trypanosomes in *Glossina*.

The two species belonging to this group (*T. vivax* and *T. uniforme*) differ from each other in dimensions.

#### xxiv. *TRYPANOSOMA VIVAX* ZIEMANN, 1905

Synonyms : *T. cazalboui* Laveran, 1906 ; *T. viennet* Lavie, 1921.

This trypanosome (Pl. II, i) measures 20–26 $\mu$  in length. It occurs in cattle, sheep, goats and horses, causing a disease known as Souma which varies in severity and may be fatal. *T. vivax* is also found in antelopes which act as reservoir hosts of the infection.

*T. vivax* is essentially an African trypanosome, being widely distributed in most parts of tropical Africa, where tsetse-flies are found. From Africa this trypanosome has been introduced with imported cattle into various other countries and has established itself in bovines in Mauritius, West Indies, Central and South America, where—in the absence of tsetse-flies—the disease is transmitted mechanically by other blood-sucking insects, especially horse-flies (*Tabanus*).

#### *T. vivax* in Man

On one or two occasions human infections with *T. vivax* have been reported from Africa. If these observations are correct, it would seem that in exceptional cases trypanosomes of ungulates may gain a footing in man and produce a transient infection. This circumstance lends indirect support to the possible origin of *T. gambiense* and *T. rhodesiense* from *T. brucei*, which has been discussed above.

xxv. *TRYPANOSOMA UNIFORME* BRUCE *et al.*, 1911

This species (Pl. II, k) differs from *T. vivax* only in dimensions, its length being 12–20 $\mu$ . It occurs in cattle, buffalo and sheep, as well as in antelopes, causing in livestock a disease similar to that produced by *T. vivax*.

The distribution of *T. uniforme* is more limited than that of *T. vivax*. Up to the present it has been recorded only from East and Central Africa (Uganda and Belgian Congo).

TRYPANOSOMES OF THE *CONGOLENSE* GROUP

The trypanosomes belonging to this group (see Table 9) are the most important ones affecting domestic animals in Africa.

The two species comprising this group are generally polymorphic, differing in dimensions and in the presence or absence of a free flagellum. They have a medium-sized kinetoplast—intermediate between that in trypanosomes of the *brucei-evansi* group and that in trypanosomes of the *vivax* group—which typically occupies a marginal position. The undulating membrane may be conspicuous or inconspicuous (Pl. II, l–p).

Trypanosomes of the *congolense* group are transmitted by various species of *Glossina*. Development in the tsetse-fly takes place in the intestine and in the proboscis, while the salivary glands are never invaded. The trypanosomes taken up with the blood of the mammalian host at first develop in the midgut of the fly, where they assume the form of long trypanosomes devoid of a free flagellum. These flagellates migrate into the proventriculus and thence into the proboscis, where they give rise to crithidial forms, which likewise have no free flagellum. These forms attach themselves to the walls of the proboscis, later migrating into the hypopharynx, where they are transformed into metacyclic trypanosomes which are similar in structure to some of the blood-forms (see Fig. 27 and Table 11).

The *congolense* group comprises at least two species (*T. congolense* and *T. simia*) but possibly more.

xxvi. *TRYPANOSOMA CONGOLENSE* BRODEN, 1904

Under the name *T. congolense* are united several variants or strains, the relationship of which is not quite clear and which might

possibly represent separate species. We shall consider here two of the most common variants.

One of these variants (Pl. II, l) is typically monomorphic and is characterized by small forms, measuring 9–18 $\mu$  in length and having no free flagellum or only a very short one.

The other variant appears to be dimorphic, for in addition to the short forms described above, there occur long forms having no free flagellum and measuring up to 25 $\mu$  in length (Pl. II, p). In both variants the undulating membrane is feebly developed, or inconspicuous.

The monomorphic trypanosomes correspond to the classical type of *T. congolense*, while the dimorphic forms have been referred to a separate species, under the name *T. dimorphon* Laveran & Mesnil, 1904. It is possible, however, that the different forms assumed by these trypanosomes are influenced by the kind of host in which they happen to be living. The classification of these trypanosomes stands in need of revision, but in the meantime it is best to retain both variants within the species *T. congolense*.

*T. congolense* has a wide distribution throughout the greater part of tropical Africa. It is pathogenic to various domestic animals (equines, cattle, goats, sheep and pigs) in which it usually produces a chronic disease varying in severity. It is also infective to most laboratory rodents and occurs naturally in antelopes, which represent its reservoir hosts.

#### xxvii. *TRYPANOSOMA SIMIÆ* BRUCE *et al.*, 1912

Trypanosomes of this species are highly polymorphic, being represented by the following three types: (a) long stout forms with a conspicuous undulating membrane, constituting the great majority (90 per cent.) (Pl. II, n); (b) long slender forms with an inconspicuous undulating membrane (7 per cent.) (Pl. II, o); and (c) short forms (3 per cent.), indistinguishable from the corresponding forms of *T. congolense* (Pl. II, l). The great majority of these trypanosomes are devoid of a free flagellum, but a small percentage of the long forms may have a short free flagellum (Pl. II, m). Their measurements vary from 12 to 24 $\mu$  in length.

*T. simiæ* has been reported chiefly from East Africa and the Belgian Congo, but it also occurs in some parts of West Africa. The animals

chiefly affected are domestic pigs, to which this trypanosome is extremely pathogenic, causing a fatal disease with an acute course, usually lasting only a few days. It has also been reported from horses and cattle. Under experimental conditions *T. simia* is slightly virulent to goats and sheep but not infective to laboratory animals, except monkeys to which it is sometimes very pathogenic. Under natural conditions *T. simia* occurs in the warthog, which is its reservoir host.

From the available epidemiological data it would seem that most outbreaks of acute pig-trypanosomiasis are due to mechanical transmission by blood-sucking insects other than *Glossina* (probably horse-flies) after *T. simia* has been introduced into the herd by a few tsetse-flies which had derived their infection from warthogs.

### TRYPANOSOMES OF THE LEWISI GROUP

The general characteristics of trypanosomes of the *lewisi* group are given in the "Key" in Table 9, under Section A.

The only species of medical interest in this group is *T. cruzi*, the trypanosome causing Chagas' disease or American human trypanosomiasis, but a description will also be given of three species occurring in lower animals. One of these, the rat trypanosome, *T. lewisi*, has been recorded from man, while another, *T. theileri*, is common in ruminants. In view of the occurrence of the latter species in wild and domestic ruminants of Africa, it is important to differentiate it from the tsetse-borne trypanosomes encountered in the same hosts. The third species, *T. grayi*, which is a natural parasite of the African crocodile, presents another problem. Since the intermediate host of *T. grayi* is *Glossina palpalis*, the possible presence of its developmental stages should be taken into consideration when examining wild tsetse-flies for infections with mammalian trypanosomes.

#### xxviii. *TRYPANOSOMA GRAYI* NOVY, 1906

Among the tsetse-borne trypanosomes mention should be made of *Trypanosoma grayi* which occurs in the blood of the African crocodile (*Crocodilus niloticus*) and is transmitted by *Glossina palpalis*. Since the developmental stages of this trypanosome

are liable to be encountered in tsetse-flies in the course of dissections carried out in epidemiological surveys, their presence might lead to confusion with trypanosomes of medical and veterinary importance. In such cases it is essential that the crocodile trypanosome should be recognized and differentiated from the mammalian parasites.

*T. grayi* is one of the largest trypanosomes, the blood forms measuring about  $90\mu$  in length. In general appearance it resembles the cattle trypanosome, *T. theileri* (Pl. II, q)—described below—from which it differs in its greater length, the situation of the kinetoplast close behind the nucleus and a more highly developed undulating membrane. The posterior end of the body is drawn out to a point and there is a long free flagellum. The infection in the crocodile is so slight that the trypanosomes are rarely detected in its blood.

In the tsetse-fly (see Fig. 27 and Table 11) the trypanosomes first develop in the midgut, giving rise to a mixture of trypanosome forms and crithidial forms, including long filamentar forms. These later migrate into the hindgut, where they become smaller and are eventually transformed into small metacyclic trypanosomes. The developmental forms of *T. grayi* can be distinguished from the other tsetse-borne trypanosomes by the following features: (a) the presence of crithidial forms in the midgut; (b) the presence of these and of metacyclic trypanosomes in the hindgut; and generally by the possession of (c) a large rod-shaped kinetoplast and (d) a long free flagellum.

Since the metacyclic forms develop in the "posterior station" of the tsetse-fly, transmission of *T. grayi* to the crocodile is effected by the contaminative method, the infective trypanosomes being liberated in the mouth of the crocodile—either with the faeces deposited by the fly or when the fly is crushed—and penetrating through the mucous membrane.

#### xxix. *TRYPANOSOMA CRUZI* CHAGAS, 1909

Synonym: *Schizotrypanum cruzi* (Chagas, 1909).

Relation to Disease.—*Trypanosoma cruzi* is the cause of a disease known as Chagas' disease, or American human trypanosomiasis, which may assume an acute or chronic form: the former is mani-

fested by fever, œdema, adenitis and progressive anæmia, while in the latter cardiac symptoms (parasitic myocarditis) predominate.

**Geographical Distribution.**—Chagas' disease occurs throughout South America, especially in Brazil and Argentina; in most of Central America, and in Mexico. Its actual distribution probably extends further north, for—as will be shown below—some of the lower mammals naturally infected with *T. cruzi*, as well as the vectors, have also been found in the United States, though no human cases have yet been recorded from the last-named country.

## MORPHOLOGY AND LIFE-CYCLE

### The Parasite in the Mammalian Host

In the blood of man and other mammalian hosts *T. cruzi* (Pl. II, r, s; Fig. 28, f) is monomorphic, being represented by medium-sized trypanosomes, measuring on the average  $20\mu$  in length. The body is fairly thick and in the majority of individuals it is characteristically curved in the form of a sickle or crescent, with a pointed posterior end. The nucleus is situated in the middle of the body, while the kinetoplast is subterminal. The kinetoplast, which is typically circular in outline, is considerably larger than that of any of the other mammalian trypanosomes considered above, including those of the *virax* group. Owing to its large size it is usually in contact with both sides of the body and sometimes produces a bulge at the hind end. The undulating membrane is slightly developed, producing only two or three convolutions, and a free flagellum of medium length is always present.

*T. cruzi* can be readily found in the blood only in the early stages of the disease, but becomes scarce when it assumes a chronic course.

**Reproduction.**—*T. cruzi* has a peculiar type of development in the mammalian host. It does not multiply in the trypanosome stage in the blood, like the species which have been described previously, but periodically the trypanosomes disappear from the blood and invade various tissues of the body, where they penetrate into different cells, including those of the reticulo-endothelial system and muscles. They show a special predilection for the heart muscle, which is the organ chiefly invaded by them. Having entered a muscle fibre the trypanosomes lose their flagellum, become rounded and assume the leishmanial form, which measures from

1.5 $\mu$  to 4 $\mu$  in diameter. The leishmanial forms proceed to multiply by binary fission (Figs. 28, a, b; 20, l-n) and as they increase in

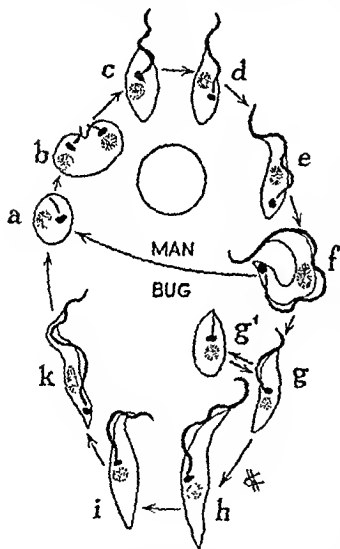


FIG. 28.—LIFE-CYCLE OF *Trypanosoma cruzi* ( $\times 2,000$ ). (After Hoare, from Broom, 1942.)

a-f, Stages in man: a, b. Leishmanial forms multiplying in muscle fibres and giving rise to (c, d) crithidia, from which (e) trypansomes develop; f. Trypanosome in the blood. g-k. Stages in the bug: g, h. Crithdial and (g¹) leishmanial forms in midgut; i. Crithdial form and (k) metacyclic trypansome in rectum. Erythrocyte drawn to scale.

numbers they destroy the adjoining tissues in which they form cyst-like agglomerations devoid of a special wall (Fig. 29). After some time the leishmanial forms become elongated, produce a flagellum and are transformed into crithdial forms (Fig. 28, c, d), which in their turn undergo binary fission giving rise to trypansome forms (Fig. 28, e). These trypansomes again enter the circulation and reappear in the peripheral blood (Fig. 28, f). This cycle, which is illustrated in Fig. 28, is repeated over and over, the trypansomes making their appearance in the blood for some time and then disappearing to invade the muscles and other cells, where they multiply. Thus, multiplication of *T. cruzi* is discontinuous or periodical, and it

does not take place in the blood in the trypansome stage but proceeds in the muscles or other tissues in the leishmanial stage.

**Cultivation.**—*T. cruzi* can be readily cultivated in N.N.N. medium

(see Chapter 19). In cultures grown at 22–24° C. the blood-trypanosome assumes forms corresponding to its stages of development in the intermediate host (Fig. 28, g–k). Owing to the ease with which *T. cruzi* can be grown *in vitro*, cultivation of the patient's blood is employed as a method of diagnosis of Chagas' disease.

### The Parasite in the Insect-Host

The intermediate hosts of *T. cruzi* are represented by blood-sucking bugs of the family Reduviidæ, numerous representatives of

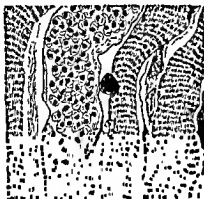


FIG. 29. — *Trypanosoma cruzi*: LEISHMANIAN FORMS IN HEART MUSCLE ( $\times 750$ ). (After Brumpt, 1936.)

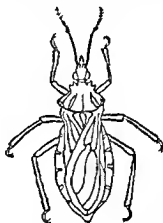


FIG. 30. — *Triatoma*: VECTOR OF CHAGAS' DISEASE. (After Alcock, 1920.)

which serve as vectors of Chagas' disease. The following are the chief genera and species responsible for its transmission: *Panstrangylus* (= *Triatoma*) *megistus* (Fig. 30), *P. geniculatus*, *Eutriatoma sardida*, *Triatoma infestans*, *Rhodnius pralixus*, *Eratyrus cuspidatus*.

The development of *T. cruzi* takes place exclusively in the alimentary canal of the bug (Fig. 31), all stages of which (larva, nymph and imago) are susceptible to infection. When ingested with infected blood the trypanosomes pass into the midgut where the blood-forms (Fig. 28, f) are transformed into erithidial forms (Fig. 28, g, h). These multiply rapidly and gradually extend to the hindgut, represented in the bug by the rectum alone, where smaller erithidial forms (Fig. 28, i) and finally the infective metacyclic



trypanosomes (Fig. 28, k) are produced. The latter are similar in appearance to the blood-forms but have a more slender body.

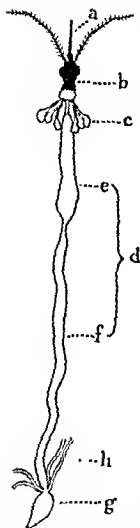


FIG. 31. — ALIMENTARY TRACT OF REDUVIID BUG (*Triatoma*). (Adapted from Pinto, 1942.)

a. Proboscis; b. Pharynx; c. Salivary glands; d. Midgut, comprising stomach (e) and intestine (f); g. Rectum; h. Malpighian tubes.

The entire cycle of development in the bug occupies from 6 to 15 days, according to the stage of the insect. Among the developmental forms in the gut of the bug are also found leishmanial forms (Fig. 28, g') but these constitute an inconstant phase and do not represent an essential stage in the life-cycle of the trypanosome.

Under experimental conditions *T. cruzi* is capable of developing in bed-bugs (*Cimex* spp.) and in some ticks. The development in the bed-bug is similar to that in the natural vectors, and metacyclic trypanosomes recovered from the faeces of the former are infective to various laboratory animals. Furthermore, it has recently been shown that in European laboratories, where strains of *T. cruzi* are maintained in these animals, bed-bugs were responsible for the spontaneous transmission of the infection to monkeys kept in the same room.

**Method of Transmission.**—From the foregoing description it is seen that the infective forms of *T. cruzi* are produced in the hindgut or "posterior station" of the vector. The method of transmission of this trypanosome is, therefore, quite different from that of the African human trypanosomes. While in the latter transmission is *inoculative*, through the bite of the tsetse-fly, in *T. cruzi* transmission is *contaminative* and is effected when the faeces of the bug, containing the metacyclic trypanosomes, are deposited on some mucous membrane (e.g. conjunctiva, mouth, nose).

This method of transmission is facilitated by the habits of the Reduviid bugs, which usually defecate while feeding on the mammalian host. It has been calculated that the droppings of

infected bugs may contain up to 3,500 metacyclic trypanosomes per 1  $\mu$ l. Furthermore, unlike the tsetse-flies, the Reduviid bugs are very efficient vectors of *T. cruzi*, for in these insects the infection rate may be as high as 100 per cent. Owing to this circumstance, these insects can be used for diagnostic purposes, as described below.

In the endemic areas of Chagas' disease the Reduviid bugs commonly occur in large numbers in the primitive native dwellings, where they spend the day hidden in cracks of the walls, ceilings, etc. In the night they emerge into the open and attack the sleeping inmates, chiefly in the face and lips (hence their name *barbeiros*, or barbers, in Brazil, and "kissing bugs" in the United States). While feeding the bugs habitually void their fæces. Under these conditions infection can be produced by the droppings either contaminating the mucous membranes of the mouth, nose or eyes directly, or being introduced into the skin by scratching and crushing the bugs, especially if an abrasion is present.

### HOST-PARASITE RELATIONSHIP

**The Disease.**—Chagas' disease occurs mainly among infants under the age of three, but older children and adults are also occasionally affected. The infection frequently produces no symptoms or lesions but when it does the course of the disease may be acute or chronic. In acute cases there is fever and œdema, accompanied by anæmia. However, the heart is the chief organ affected, owing to the fact that the trypanosomes have a special predilection for the heart muscle, in which their multiplication—accompanied by destruction of the tissue—takes place. In such cases death may result from parasitic myocarditis. If the acute infection does not terminate fatally, it may subside in a few weeks with the disappearance of the symptoms. On the other hand, it may develop into a chronic condition, manifested by cardiac symptoms with myocardial degeneration and sometimes leading to death as the result of heart failure.

Though the total number of clinical cases recorded is only slightly above two thousand, recent epidemiological surveys have shown that the actual incidence of infection is much higher.

**Course of Infection.**—When a bug deposits infected fæces on the

surface of a mucous membrane the metacyclic trypanosomes actively penetrate through it and invade cells of the subcutaneous reticulo-endothelial system. In the macrophages the trypanosomes are transformed into leishmanial forms (Fig. 28, a, b) and multiply locally. At the point of entry there appears a local reaction in the form of a swelling, known as "*chagoma*," which is due to an inflammatory exudation in the subcutaneous tissues occupied by the parasites. The most common portal of entry of the parasites is the conjunctiva, the invasion of which is manifested by a unilateral swelling of the eyelids, known as "*Romana's sign*." After multiplying for some days locally in the subcutaneous tissues the leishmanial forms give rise to trypanosomes (Fig. 28, e, f) which invade the blood stream, whence they are carried to various inner organs and tissues in which they continue their development, producing the characteristic symptoms of the disease. These may appear from 4 to 20 days after the initial infection (incubation period).

*T. cruzi* is usually present in the blood in appreciable numbers only in acute cases, during the first 3 or 4 weeks of the infection, but from the fifth day onwards, when the trypanosomes retreat from the blood to multiply in the muscles and other tissues, their release into the general circulation takes place at irregular intervals and in progressively diminishing numbers. In chronic cases it may be difficult to detect the parasites by direct examination of the blood, as they are confined chiefly to the inner organs, especially the heart. The pathological effect of Chagas' disease is due mainly to destruction of the reticulo-endothelial cells and degeneration of the cardiac muscles resulting from the proliferation of the parasites. When the central nervous system is involved the trypanosomes are found in the cells of the neuroglia and provoke meningo-encephalomyelitis.

**Immunity.**—The question of immunity in Chagas' disease has not been adequately studied. It would seem that in endemic areas the inhabitants, who are exposed to repeated infections, develop partial immunity or premunition, becoming carriers of *T. cruzi*, which is rarely found in the blood but is confined to the tissues. It is possible that such carriers frequently escape detection and consequently the actual incidence of Chagas' disease might be much higher than the figures based on existing records.

## Infection in Lower Mammals

*T. cruzi* is infective to a wide range of mammals under both experimental and natural conditions. The existence of natural animal hosts of this trypanosome is of considerable epidemiological importance, as will be shown below.

**Experimental Infection.**—*T. cruzi* can be successfully inoculated into a number of laboratory animals, both by depositing the infected droppings of Reduviid bugs on the mucous membrane and by injecting the blood of the mammalian host. The course of the infection in these animals varies according to the strain and virulence of the trypanosomes used. The virulence may vary in the course of serial passages and the infection may be acute or chronic, with trypanosomes appearing in the blood and disappearing from it at irregular intervals, as in the case of naturally infected hosts. The inoculation of laboratory animals can be used as a diagnostic method for the detection of *T. cruzi* in suspected cases of Chagas' disease, the susceptibility of these animals varying in the descending order as follows: pups and kittens, white mice, guineapigs. Monkeys (*Macacus*, *Cercopithecus* and marmosets) are also susceptible.

**Natural Infection.**—In various countries of the New World numerous wild and domestic mammals, belonging to about 40 species, have been found naturally infected with *T. cruzi*. The most important of these are armadillos (*Dasypus*) in South America; opossums (*Didelphis*) in South America, Panama and the United States; the wood rat (*Neotoma*) in U.S.A.; dogs, cats and pigs in South America.

**Reservoir Hosts.**—The finding of *T. cruzi* in animals is of considerable importance in the epidemiology of human trypanosomiasis. The part played by different hosts varies in this respect, for while the domestic animals live in close proximity to man, some of the wild animals only occasionally come in contact with man. The occurrence of natural infections in wild animals is closely correlated with the incidence of *T. cruzi* among Reduviid bugs. Infected bugs belonging to numerous species are encountered throughout the Americas—from the southern part of the United States to Argentina—not only in localities where the human disease is endemic but also outside these areas, where they inhabit the burrows of arma-

xxx. *TRYPANOSOMA LEWISI* (KENT, 1880)

*Trypanosoma lewisi* is a non-pathogenic parasite of rats, various genera and species of which—including the common brown and black rats—harbour it throughout the world.

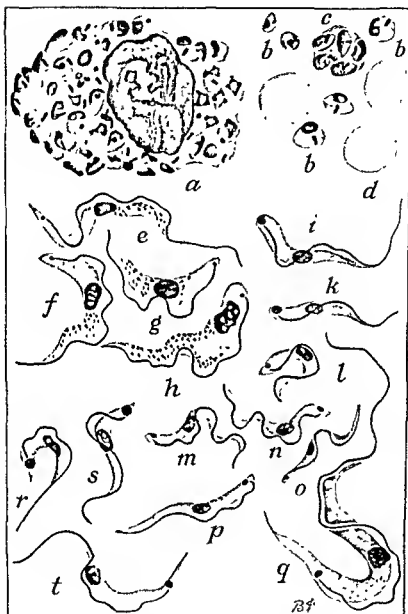
The body of this trypanosome (Pl. II, t) is elongated and drawn out to a point at the posterior end. The large kinetoplast is situated at some distance from this end, while the nucleus lies in the anterior half of the body. The undulating membrane is inconspicuous and a long free flagellum is present. *T. lewisi* measures about  $25\mu$  in length. In the blood of the rat the development of *T. lewisi* proceeds as follows. During the first 8–10 days of the infection it multiplies in the crithidial stage by multiple fission, in the course of which an individual divides several times in succession without complete fission of the cytoplasm, with the result that the daughter-individuals remain attached to each other for some time before breaking off and becoming trypanosomes again. At the end of this period reproduction comes to an end and only the typical "adult" trypanosomes are left in the blood, where they persist for some time (1–4 months).

The intermediate host of *T. lewisi* is the rat-flea (*Ceratophyllus fasciatus*). When ingested with the rat's blood the trypanosomes first develop in the midgut where they multiply, giving rise to a special kind of trypanosome form. These later pass into the rectum, where they are transformed into crithidial forms, which in their turn give rise to small metacyclic trypanosomes. This terminates the life-cycle of *T. lewisi* in the flea, which lasts about 5 days.

Transmission is effected through the faeces of infected fleas when voided on the body of the rat. Infection takes place through the mouth, when the rat licks its fur which is contaminated with the droppings of the flea.

*T. lewisi* in Man

A case of human infection with *T. lewisi* was recorded from a four-months-old Malay child, who was suffering from fever, with numerous trypanosomes in its blood. The trypanosomes disappeared after the fever had abated. Local rats also harboured this trypanosome but all attempts to infect clean rats from the child failed. This is the only case of its kind known. Though it is suspected that the infection was acquired from rats, its origin is still doubtful.



THE HEMOFLAGELLATES. (X 2000).

(Original, and after Wenyon, 1926)

a, b, c *Leishmania donovani* and *L. tropica* in tissue smears. a Macrophage packed with parasites; b Parasites scattered by rupture of host-cells, c Detached portion of host-cell with parasites, d Erythrocytes e-t. Trypanosomes. e-h *Trypanosoma gambiense*, *T. rhodesense* and *T. brucei* e Slender, f Intermediate, g Stumpy, h Posterior-nuclear forms, e, f *T. etansi* and/or *T. equiperdum*; i *T. vivax*; k. *T. uniforme*; l+p. *T. congolense*; l+m+n+o *T. simiae*; q *T. theileri* (?=*T. traglephii*), r, s *T. cruzi*; t *T. lewisi*.

To face page 216



xxx. *TRYPANOSOMA THEILERI* LAVERAN, 1902

*Trypanosoma theileri* (Pl. II, q) occurs in domestic cattle and in wild bovines in most parts of the world. It is one of the largest mammalian trypanosomes, usually measuring  $60-70\mu$  in length, though smaller forms are also encountered. The posterior end of the body is drawn out to a point; the nucleus is more or less central; the kinetoplast, which is large, is situated at some distance from the posterior extremity, sometimes near the nucleus; the undulating membrane is fairly conspicuous, and there is a long free flagellum. *T. theileri* multiplies in the blood by unequal binary fission in the crithidial stage.

*T. theileri* appears to have no harmful effect upon its host and is usually present in small numbers in the blood of cattle, but since the parasite may increase considerably in numbers when the host is weakened by disease, such as piroplasmiasis or rinderpest, some observers have attributed pathogenic properties to this trypanosome.

The intermediate host of *T. theileri* is represented by Tabanid flies, in which the trypanosomes complete their development in the "posterior station," transmission being effected by the contaminative method.

Trypanosomes similar to *T. theileri* also occur in various African antelopes. One of these, described as *T. tragelaphi* is practically indistinguishable from *T. theileri* and is probably identical with it, while another, *T. ingens*, is considerably larger, measuring from  $72\mu$  to  $122\mu$  in length, and might possibly represent an independent species.



## CHAPTER 12

### THE MALARIA PARASITES

THE malaria parasites are blood-inhabiting protozoa belonging to the class SPOROZOA, order Hæmosporidia and family Plasmodiidae. They are all included in the genus *Plasmodium*, species of which are parasitic not only in man but also in apes, monkeys, birds and some other vertebrate animals.

**Relation to Disease.**—Four species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) are pathogenic to man, causing malaria, which is an acute or chronic disease characterized by a succession of attacks (primary and relapses) with periodic paroxysms of fever (typically intermittent), and associated with anæmia and splenomegaly.

Each of the four species of *Plasmodium* gives rise to a different type of malaria, as follows:—

- (1) *P. falciparum* causes Malignant Tertian, Subtertian, Æstivo-Autumnal or *falciparum* malaria ;
- (2) *P. vivax* causes Benign Tertian or *vivax* malaria ;
- (3) *P. malariae* causes Quartan or *malariae* malaria ;
- (4) *P. ovale* causes *ovale* Tertian malaria.

### MORPHOLOGY AND LIFE-CYCLE

Malaria parasites undergo a complicated cycle of development with an alternation of hosts, one of which is a vertebrate, the other a mosquito. In the case of plasmodia causing human malaria, the vertebrate host is man, while the invertebrate is a mosquito of the genus *Anopheles* (Fig. 37). In the human body the parasites live chiefly within the red blood corpuscles, in which the asexual cycle of their development takes place. When certain stages of these parasites are taken up by a mosquito with the blood meal, they undergo in the body of this insect the sexual cycle of development which terminates in the production of the infective forms (SPOROZOITES). When these are introduced into the human organism through the bite of an infected mosquito they initiate in man a new malarial infection. Except for the presence of two hosts, the life-cycle of the malaria parasites is similar to that of the coccidia (Chapter 6), to which they are closely related.

Since there is a close similarity in the general appearance and life-cycles of the malaria parasites both in the human and in the insect hosts, an account of the life-history of one of them can serve to illustrate the course of development of all the four parasites, except for minor details which are dealt with in the descriptions of the separate species.

### The Parasite in the Human Host

Malarial infection is produced through the bite of a female Anopheline mosquito harbouring in its salivary glands the sporozoites of *Plasmodium*. When the insect feeds on man, the sporozoites are injected into the skin and proceed to develop in the new host. The behaviour of the sporozoites in the human body is not definitely known. It was formerly thought that they invade the erythrocytes directly and develop in these cells exclusively. However, recent observations indicate that the parasites commence their development outside the blood stream and only later invade the red blood corpuscles. From the available evidence it would seem that on inoculation by the mosquito the sporozoites first enter the circulation, where they remain not longer than 60 minutes, after which they disappear from the blood. Their initial development presumably proceeds in the tissue cells for several days, after which the parasites begin to invade the erythrocytes and continue to develop in them throughout the subsequent course of the infection.

In the case of human malaria, developmental forms of the parasites outside the red cells, or the EXOERYTHROCYTIC stages, have so far been demonstrated only in *P. vivax*, the evidence of their presence in other species being circumstantial, based chiefly on the existence of a negative period—when the blood is free of parasites—intervening between the inoculation of sporozoites and the appearance of parasites in the blood. However, the complete course of development of the exoerythrocytic stages has been studied only in the case of some malaria parasites of birds. The best example is afforded by *P. gallinaceum* of the domestic fowl, the development of which proceeds as follows. After having been introduced into the skin of a chicken the spindle-shaped sporozoite (Fig. 33, a) penetrates into a cell of the reticulo-endothelial system and becomes rounded (Fig. 33, b). The parasite then begins to multiply by a process of schizogony in the course of which it grows and the

nucleus divides repeatedly, giving rise to numerous daughter-nuclei (Fig. 33, c, d). Finally the schizont undergoes segmentation, producing daughter-individuals, or merozoites, the number of which corresponds to that of the nuclei present. The host-cell finally ruptures, thus liberating the merozoites (Fig. 33, e). The development of the first generation of parasites occupies about 42 hours. The merozoites then attack new macrophages in which a second generation develops in the same way. After this exoerythrocytic development has proceeded for about 75 hours, involving reticulo-endothelial cells of the skin, spleen, heart, kidney and brain, the merozoites begin to invade the erythrocytes (Fig. 33, f) in which they also multiply by schizogony (Fig. 33, g, h). From then onwards the number of parasitized red cells increases progressively with each successive segmentation of the exoerythrocytic forms. The infection in the bird is maintained by both exoerythrocytic and erythrocytic multiplication, which co-exist and are probably interchangeable, the former constantly giving rise to the blood forms, while the latter may revert to tissue forms. A distinction is made between the exoerythrocytic stages which develop directly from the sporozoites and precede the invasion of the erythrocytes, on the one hand, and the exoerythrocytic stages of development which persist in the host after the erythrocytic cycle has been established, on the other hand. The former are known as the PRIMARY EXOERYTHROCYTIC or PRE-ERYTHROCYTIC forms, while the latter are described as the SECONDARY EXOERYTHROCYTIC forms. One of the main features distinguishing the exoerythrocytic and erythrocytic parasites is the absence of PIGMENT in the former and its presence in the latter.

There is reason to believe that the initial development of the human malaria parasites is similar to that described above but differing in details. According to this hypothesis, in *P. falciparum* the pre-erythrocytic development lasts about 7 days, while in *P. vivax* it lasts about 9 days. Furthermore, from the available evidence it would seem that the exoerythrocytic development of *P. falciparum* is restricted to the pre-erythrocytic phase, whereas in *P. vivax* it passes into the secondary phase and persists after the parasites have gained access to the blood stream. Pre-erythrocytic stages of schizogony of the last-named species (Fig. 32, EE) have recently been demonstrated in the liver of a volunteer experimentally infected with sporozoites.

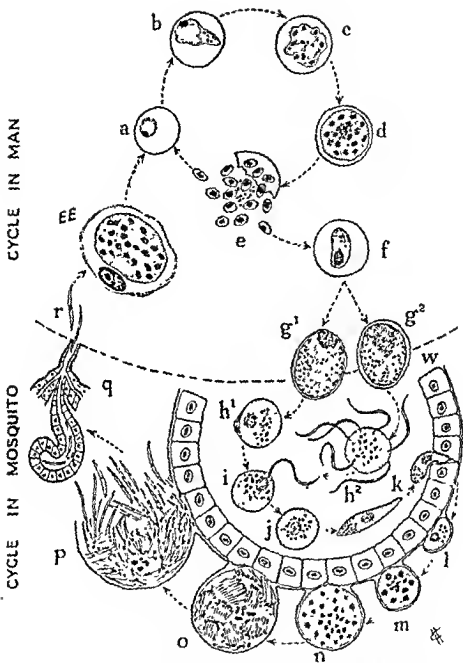


FIG. 32.—LIFE-CYCLE OF MALARIA PARASITE (*Plasmodium vivax*).  
(Original.)

In the blood the parasites develop exclusively within the erythrocytes which are initially invaded by merozoites produced by schizogony of the primary exoerythrocytic forms. The youngest stages in the blood are small rounded forms (Fig. 32, a) containing a large vacuole which displaces the cytoplasm to the periphery of the body, while the nucleus is situated at one of its poles. In optical section the cytoplasm is seen as a circle surrounding a vacuole, on account of which the young parasites have an annular appearance and are known as RING-FORMS. As these forms grow they gradually increase in size and may become more irregular in shape (Fig. 32, b). The growing stages of the parasite (including the ring-forms) are known as TROPHOZOITES. In the course of their development in the red blood corpuscles the parasites feed at the expense of the hæmoglobin from which they absorb the protein (globin) fraction, leaving—as a product of metabolism—a pigment representing hæmatin. The pigment is deposited in the body of the parasite in the form of characteristic dark granules which are absent in the young ring-forms but accumulate in the later stages of development. In addition to hæmoglobin, the parasites also consume glucose from the blood.

After a period of growth the parasite begins to multiply by schizogony which proceeds as follows. The nucleus of the SCHIZONT—as the parasite is now called—divides repeatedly (Fig. 32, c, d), giving rise to a variable number of daughter-nuclei. Nuclear division is followed by segmentation of the cytoplasm of the schizont, as the result of which small rounded uninucleate forms known as MEROZOITES are produced, their number corresponding to the number of nuclei in the mature schizont, or SEGMENTER, and differing according to the species of *Plasmodium*. After segmentation is completed the red blood corpuscle bursts and the merozoites are liberated into the blood stream together with the pigment left over from the schizont (Fig. 32, e). The merozoites then enter new erythrocytes in which another generation of the parasite is produced by schizogony. As regards the pigment which is released into the blood, it is phagocytosed by leucocytes and by endothelial cells of the capillaries, being ultimately deposited in various organs and tissues, especially in the spleen and bone-marrow. The asexual cycle of development is repeated over and over again in the course of the infection, leading to a progressive increase in the number of

parasites in the blood (parasitæmia) until their multiplication is checked by the defence forces of the host. The asexual stages of the four human malaria parasites differ from each other morphologically and in their effect upon the host-cell, as will be seen from the description of the separate species. When observed in fresh preparations on the warm stage of a microscope the parasites exhibit amœboid movements.

Though typically each host-cell harbours a single parasite, sometimes more than one may be present. Such MULTIPLE INFECTIONS usually occur among the young trophozoites (ring-forms). Occasionally, instead of occupying the middle of the erythrocyte, as is usual, the young parasite is flattened against the periphery of the cell, producing so-called MARGINAL or ACCOLÉ forms. Multiple infections and marginal forms are especially common in *P. falciparum* (see Pl. III).

In the course of the development of the parasite the host-cell may undergo certain changes. Thus, in the case of *P. vivax* and *P. ovale* the erythrocyte becomes enlarged. Furthermore, in these species and in *P. falciparum* it acquires a stippling with characteristic spots which are thought to represent the result of colloidal disintegration and precipitation within the erythrocyte.

The length of the asexual cycle is known as the *periodicity* of development. It differs in various types of malaria, being 48 hours in the tertian forms and 72 hours in the quartan form. The multiplication of the parasites is more or less synchronized, therefore at any given time most of the parasites are in the same stage of development. However, in the earlier phases of the infection there may be groups or "broods" of the same generation developing at different times, with the result that several stages of the parasite occur in the blood simultaneously. As will be shown later, the stages of development of the parasite are closely correlated with the periodicity of febrile paroxysms in the patient.

After several generations of merozoites have been produced, some of these give rise to sexually differentiated forms known as GAMETOCYTES, while others continue the asexual cycle of development. On entering new red blood corpuscles the former proceed to grow (Fig. 32, f) and reach the size of a schizont but without division of the nucleus, which remains single in the gametocytes. As a rule this development proceeds in the vessels of the spleen and bone-marrow, ✓

therefore young gametocytes are not commonly found in the peripheral blood. The appearance of mature gametocytes varies in the different species: while in *P. falciparum* they are elongated and curved in the form of a crescent, in the other species they are rounded. The female or macrogametocyte (Fig. 32, g<sup>1</sup>) has deeply staining cytoplasm and a small compact nucleus, whereas the male or microgametocyte (Fig. 32, g<sup>2</sup>) has faintly staining cytoplasm and a large diffuse nucleus. This difference in the staining reactions of the cytoplasm is due to the fact that the female gametocyte contains reserve food material which takes up a deep blue colour when stained by the Romanovsky methods. As regards the nucleus, it contains more chromatin in the male gametocyte because of the greater demand for nuclear material in the course of its subsequent transformation into microgametes. The abundance of nuclear substance also accounts for the pinkish tint which the cytoplasm of the microgametocytes commonly assumes (Pl. III). In this and in other respects the female and male forms of *Plasmodium* are comparable to the sex cells of higher animals. Like the trophozoites and schizonts, the gametocytes contain pigment granules. The gametocytes do not undergo any further development in the human body, in which only the asexual cycle persists, from time to time giving rise to new broods of gametocytes.

Human malaria parasites can be cultivated at 37° C. in a medium containing glucose but their growth in culture does not proceed beyond one or two asexual generations.

### The Parasite in the Insect-Host

The further development of the gametocytes, representing the sexual cycle of the parasite, takes place in the midgut, or stomach, of a female Anopheline mosquito, after they have been ingested together with the blood of an infected human being. The asexual forms, which reach the stomach together with the blood meal, rapidly perish, the mature sexual forms being the only ones capable of continuing the life-cycle. In the stomach of the mosquito the erythrocytes harbouring the gametocytes are ruptured and the gametocytes themselves escape into the lumen and proceed to develop further. In the case of the male gametocyte, the nucleus breaks up into a number of separate chromatin granules, while from the surface of the cytoplasm are given off several long thin processes,





chromatin, it is transformed into the mature female gamete, or MACROGAMETE, the nucleus of which moves to the surface of the body, which at this point is slightly elevated (Fig. 32, h<sup>1</sup>). When a microgamete comes in contact with a macrogamete, to which it is probably attracted by chemotaxis, it penetrates into it and fertilization takes place (Fig. 32, i), the male and female nuclei fusing and the product of this union becoming a ZYGOTE (Fig. 32, j). The microgamete and macrogamete are homologous to the spermatozoon and ovum respectively of higher animals, while the zygote is comparable to the fertilized ovum. Fertilization usually takes place in from 20 minutes to 2 hours after ingestion of the parasites by the mosquito. The zygote is at first a motionless globular body but within about half an hour of its formation it becomes elongated, giving rise to a vermiform stage known as the OOKINETE (Fig. 32, k). The ookinete, which is actively motile, glides about among the intestinal contents. Its body, measuring 18–24 $\mu$  in length, is broader at one end, which contains the pigment granules, and narrower at the other end, which is directed forwards during locomotion, while the nucleus is situated in the middle of the body. The ookinetes actively force their way between the epithelial cells of the stomach until they reach the outer surface of its wall, where they settle down under the elastic membrane covering the stomach. Here they become rounded into a small sphere which is invested in a membrane formed partly by the parasite itself and partly by the elastic lining of the stomach (Fig. 32, l). This transformation takes place about 48 hours after infection of the mosquito. In this position the parasite, now known as the OOCYST, undergoes development by sporogony.

The oocysts gradually increase in size until they reach about 40–80 $\mu$  in diameter. If the intestine of an infected mosquito is examined, the oocysts appear on the surface of the stomach as transparent globular bodies (Fig. 42, b, c) in which the pigment is clearly visible. As the oocysts grow their nucleus undergoes repeated divisions, while the cytoplasm becomes vacuolated and sponge-like in structure, with the nuclei distributed all over the surface of the trabeculae. Gradually finger-like processes—each accompanied by a nucleus—are given off from the periphery of the cytoplasm. These processes become elongated and finally break off, leaving behind them residual portions of the cytoplasm (Fig.

32, m-p). The daughter-individuals, resulting from sporogony and known as **SPOROZOITES**, represent motile spindle-shaped bodies, measuring about  $15\mu$  in length, with pointed ends and a centrally situated nucleus. Later the oocyst bursts and liberates the sporozoites into the body cavity of the mosquito (Fig. 32, p) whence they wander through the body until they reach the salivary glands lying in the anterior part of the thorax (Figs. 32, q ; 42). They then

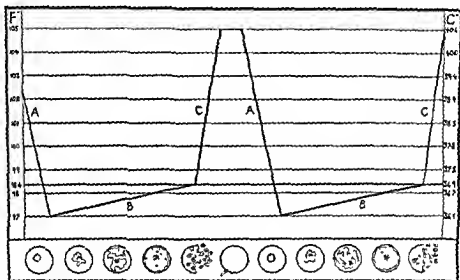


FIG. 34.—CORRELATION BETWEEN TEMPERATURE CURVE IN MALARIA AND ASEYUAL CYCLE OF DEVELOPMENT OF THE PARASITE. (After Wenyon, 1926.)

the merozoites have entered  
is the parasites grow, and  
schizonts have segmented

actively penetrate through the walls of the glands and settle within the salivary cells and in the ducts. With the invasion of the salivary glands the life-cycle of the malaria parasite is completed and the mosquito becomes infective to man. When it bites a human being, the sporozoites, which represent the infective stage of the parasite, are injected into the wound and initiate a new infection (Fig. 32, q, r), with the resumption of the asexual cycle of development in man.

The duration of the development of *Plasmodium* in the mosquito differs according to the species of parasite and vector, and varies inversely with the external temperature. At temperatures between

21° C. and 27° C. (70–80° F) it may take about 10 days. Below 16° C. (61° F.) development is arrested and remains dormant until the temperature rises again, when the parasites resume and complete their development. In this way hibernating mosquitos may carry the infection through the winter and transmit it in the spring.

The total number of oocysts present on a single mosquito stomach varies considerably, according to the number of gametocytes ingested by the vector from the human host and other factors. As a rule, there are from 20 to 30 oocysts but exceptionally much larger numbers are present. The oocysts of the different malaria parasites differ in the appearance of the pigment granules and in the pattern on which they are arranged. These characters are dealt with in the description of the separate species. Each oocyst may give rise to several thousand sporozoites, while the salivary glands frequently harbour as many as 60,000 sporozoites. It has been reckoned that this supply of sporozoites may be used up in the course of twenty blood meals covering a period of about 2 months, after which the mosquito loses its infectivity.

### SYSTEMATIC DESCRIPTION OF THE MALARIA PARASITES

In the preceding section a general account was given of the stages of development of the malaria parasites throughout their life-cycle in the human and insect-hosts, which is similar in all the four species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*). A separate description will now be given of the structure, development and bionomics of each of these species, followed by their differential diagnosis. The most important stages of these parasites are illustrated in Pl. III. The description which follows is based on the appearance of the parasites as seen in blood films and tissue smears stained by one of the Romanovsky methods (see Chapter 17), in which the cytoplasm of the parasite is coloured blue, while the nuclear elements are red and the erythrocyte is pale pink.

#### xxxii. *PLASMODIUM FALCIPARUM* (WELCH, 1897)

*Plasmodium falciparum* is the most important malaria parasite, causing Malignant Tertian, Subtertian or Æstivo-Autumnal malaria, which usually runs an acute course and frequently terminates fatally.

*Falciparum malaria* is responsible for about 50 per cent. of all cases in the world. It is restricted to warm and tropical countries (hence the name *tropical malaria* in some European countries), its distribution being limited by the mean summer isotherm of  $21^{\circ}\text{C.}(70^{\circ}\text{F.})$ , which in the Northern hemisphere lies in latitudes south of  $45^{\circ}\text{N.}$  The incidence of the disease is highest in late summer and early autumn.

Though schizogony in *P. falciparum* is completed in 48 hours and the periodicity of asexual development is therefore typically of the tertian type, in this species there frequently occur two or more broods of parasites, the segmentation of which is not synchronized, with the result that the periodicity of symptoms in the patient—which are dealt with later—is commonly irregular, especially in the early phases of the disease.

The parasites first appear in the blood of the patient 7 days after the infective bite of a mosquito, the intervening period presumably being occupied by the pre-erythrocytic development of the sporozoites. The young ring-forms of *P. falciparum* (Pl. III, 1st figure in top row) are very small, occupying only about one-sixth of the diameter of the red blood corpuscle; the cytoplasm appears as a very narrow blue circle surrounding the vacuole, with the nucleus at one side. In many rings there may be two chromatin granules—possibly indicating fragmentation or premature division of the nucleus—and marginal forms are fairly common. In many of the host-cells there may be several ring-forms (multiple infection). Occasionally the young trophozoites are irregular in shape: rectangular, piriform or in the form of a streak with the nucleus at one end. Although marginal forms, rings with two chromatin dots and multiple infections of the red cell occur in the other species, they are much more common in *P. falciparum* and are characteristic of this species. The ring-forms may remain in the peripheral blood up to 24 hours, in the course of which they increase in size and may occupy about one-third of the diameter of the red cell. The cytoplasm of the older trophozoites is irregularly thickened at different points of the body and the vacuole may be reduced in volume. As the rings grow there appear in their cytoplasm granules of blackish pigment. These are at first scattered but tend to form a single mass earlier than in the other species, i.e. before schizogony (Pl. III, 1st figure in 2nd row).

The succeeding stages of the asexual cycle—growth to schizonts and segmentation—do not normally occur in the peripheral blood as in other malaria parasites, except in severe pernicious cases. The ring-forms and the older trophozoites usually disappear from the peripheral circulation after 24 hours and are held up in the capillaries of the internal organs, such as brain, heart, intestine or bone-marrow, where their further development takes place. The retention of the parasites is due to the fact that in Malignant

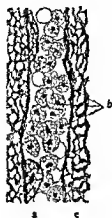


FIG. 35.—*Plasmodium falciparum* IN THE BRAIN. (After Blacklock and Southwell, 1940.)

a. Blood capillary filled with schizonts; b. Pigment in parasites; c. Brain tissue.

Tertian malaria the infected red blood corpuscles have a tendency to clump together and to adhere to the walls of the capillaries, probably as the result of the formation of a coating of fibrin on the surface of the erythrocytes. In the course of the next 24 hours the parasites in the capillaries multiply by schizogony. The trophozoite continues to increase in size, the vacuole disappears and the body of the parasite assumes a compact form. It is henceforth known as a schizont, and at first contains a single nucleus which proceeds to multiply by repeated divisions until up to 24 daughter-nuclei are present (Pl. III, 1st figure in 3rd and 4th rows). The schizont is now fully grown and occupies about two-thirds of the red cell. The pigment in the schizonts is clumped together in a mass which frequently lies at one side of the body (Pl. III, 1st figure in 3rd row). Finally the schizont undergoes segmenta-

tion, giving rise to from 8 to 24 merozoites, the average number being 16. The mature schizont of *P. falciparum* is smaller than that of any of the other malaria parasites. When the merozoites are released by rupture of the host-cell they invade new red blood corpuscles, where they are transformed into young ring-forms which then reappear in the peripheral blood. The confined space within the capillaries obviously favours the penetration of several merozoites into a single erythrocyte, hence the frequency of multiple infections in Malignant Tertian malaria. The degree of infection in this disease is considerably higher than in the other types of malaria, the density of parasites sometimes exceeding 500,000 per  $\mu$ l. (= c.mm.) of blood with up to 25 per cent. of the erythrocytes infected.

Most of the severe and fatal cases of this type of malaria are due to the blocking or occlusion of capillaries by clumps of red blood corpuscles with developing parasites, enormous numbers of which can be seen in smears and sections of *post mortem* material (Fig. 35). The distribution of the parasites in organs and tissues of the human body varies in different cases and this accounts for the diversity of clinical manifestations observed in Malignant Tertian malaria.

In Malignant Tertian malaria the infected red blood corpuscles retain their normal size throughout all the stages of development of the parasite. However, cells harbouring the older trophozoites and schizonts are frequently stippled with a few coarse red dots of irregular shape (angular, annular or comma-like), known as *Maurer's dots*, while the stroma of these cells assumes a characteristic brassy tint (Pl. III, 1st figure in 2nd row). This stippling does not affect all the host-cells and can only be exhibited in deeply stained preparations. The erythrocytes occupied by schizonts are frequently very pale, being almost colourless in stained films.

The development of the gametocytes is confined to the inner organs but sometimes the young forms may occur in the blood. The young gametocytes tend to be elongated, becoming spindle-shaped or elliptic as they grow. Finally they assume the characteristic curved shape of the mature gametocytes. These first appear in the peripheral blood after several asexual generations have undergone schizogony, usually about 10 days after the initial invasion of the blood by the parasites. Crops of gametocytes subsequently make their appearance in the blood in successive waves. The body of the mature gametocytes may be sausage-shaped, banana-shaped or crescentic, according to whether the ends are rounded or pointed, hence they are commonly referred to as *crescents*. Since the gametocytes are about one and a half times longer than the red cell harbouring them, the latter is stretched by them beyond recognition, its margin being closely applied to the convex side of the parasite and producing an arched rim across the concave side. The female and male gametocytes differ from each other in general appearance and in some structural details. The macrogametocyte (Pl. III, 1st figure in 5th row) is usually more slender and somewhat longer than the male form; its cytoplasm takes up a deeper blue colour; the nucleus is small and compact, staining dark red, while the pigment granules are closely aggregated around it. The microgametocyte

(Pl. III, 1st figure in the 6th row) is broader than the female form, being more sausage-shaped; its cytoplasm is either pale blue or tinted with pink; the nucleus, which stains pink, is large and loosely distributed, while the pigment granules are scattered in the cytoplasm around it. In both gametocytes the nucleus is situated in the middle of the body. The number of gametocytes present in Malignant Tertian malaria is variable, occasionally amounting to from 50,000

to 150,000 per  $\mu\text{l.}$  (= c.mm.) of blood, a figure which is never attained by the other species of malaria parasites. On account of their characteristic shape the gametocytes of *P. falciparum* can readily be distinguished from the rounded sexual forms of the other species.

Since schizogony normally takes place in the inner organs, in infections with *P. falciparum* only ring-forms and/or crescents are typically present in the peripheral blood, with the result that

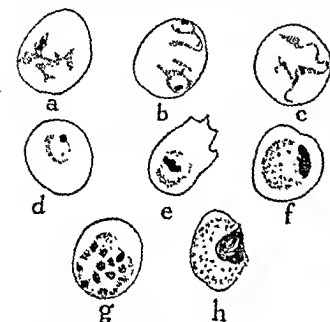


FIG. 36.—MALARIA PARASITES OF MAN AND MONKEY ( $\times 2,000$ ). (Adapted from Sinton, 1922; and Mulligan, 1935.)

a-c. *Plasmodium falciparum*: "tenue" forms. d-h. *P. knowlesi* in macaque monkey: d. Ring form; e. Mature trophozoite; f. Young schizont; g. Mature schizont; h. Microgametocyte.

blood films examined at different times may reveal one of the following pictures: (a) only ring-forms, (b) these together with crescents, or (c) crescents alone.

The sexual cycle of *P. falciparum* in the mosquito is similar to that described in the general account. Its duration depends on the external temperature, thus at  $20^{\circ}\text{C.}$  it lasts 22 days, at  $23\text{--}28^{\circ}\text{C.}$  17-18 days, and at  $30^{\circ}\text{C.}$  10-11 days. In young oocysts there are about 10-12 large black pigment granules arranged as a streak across the oocyst.

"Tenue" Phase.—In Malignant Tertian malaria there some-

times appear strains of the parasite in which the trophozoites are amœboid or irregular in shape, the cytoplasm forming irregular strands or a network, containing several chromatin masses and sometimes occupying three-quarters of the host cell (Fig. 36, a-c). Some malariologists regard such strains as belonging to a distinct species, *P. tenue*, but the majority believe that they are variants of *P. falciparum* which occur in severe infections, their appearance being the result of fusion—apparent or real—of several trophozoites in a multiple infection of the erythrocyte. Whatever the interpretation of the *tenue* strains may be, their clinical manifestations do not differ from those produced by typical strains of *P. falciparum*.

xxxiii. *PLASMODIUM VIVAX* (GRASSI & FELETTI, 1890)

*Plasmodium vivax* causes the so-called Benign Tertian form of malaria which usually runs a chronic course and is liable to relapse. *Vivax* malaria is responsible for about 43 per cent. of all cases recorded in the world and has the widest geographical distribution, occurring not only in warm and tropical countries but also in temperate regions. In general, it is found in latitudes below 43° both north and south of the equator, but in Europe it may extend to 60° N. The distribution of Benign Tertian malaria is usually limited by the mean summer isotherm of 15.5° C. (60° F.). In warm countries infections usually take place in spring and in summer.

The periodicity of the asexual cycle, which occupies 48 hours, is typically of the tertian type and the course of the development of this parasite is more closely synchronized than in the case of *P. falciparum*.

The asexual cycle of *P. vivax* (Fig. 32) involves both exocrythrocytic and erythrocytic stages, the development of which presumably proceeds along parallel lines. The pre-erythrocytic development, initiated by sporozoites injected by the mosquito, takes place in the liver, in which schizonts measuring up to 42 $\mu$  in diameter have been found (Fig. 32, EE). By the ninth day the parasites invade the erythrocytes and begin to appear in the peripheral blood. However, the exocrythrocytic development probably persists in the tissues throughout the course of the blood infection and constitutes a source from which the blood is continually re-invaded, being presumably also responsible for the production of relapses. Once



established in the blood the entire asexual cycle of development of *P. vivax* takes place in the peripheral circulation. The youngest trophozoite, or ring-form, of this species is larger than that of *P. falciparum*, occupying about one-third of the diameter of a normal red blood cell (Pl. III, 3rd figure in 1st row). The ring-forms gradually increase in size for about 36 hours. After about 6 hours the trophozoites either retain the annular form or become amœboid, with irregular outgrowths of cytoplasm. The vacuole is considerably enlarged or it is divided up into several smaller ones, while the nucleus also increases in volume and light brown pigment granules begin to appear in the cytoplasm of the parasite.

While the red blood corpuscles containing the young ring-forms retain their normal appearance, those harbouring the older trophozoites undergo characteristic changes which persist throughout all the subsequent stages of the asexual cycle. In the first place, the host-cell gradually increases in size until finally it is about  $10-11\mu$  in diameter, as compared with  $7.5\mu$  of the normal erythrocyte. It also becomes paler than the latter, and at the same time acquires a stippling with numerous bright red spots, known as *Schüffner's dots*, which are scattered throughout the cell.

As the parasite continues to grow its shape becomes still more irregular, owing to the formation of pseudopodium-like processes which spread in all directions and sometimes anastomose. Finally the fully grown amœboid trophozoite comes to occupy the greater part of the enlarged host-cell. The pigment granules are now scattered in fair numbers in the cytoplasm (Pl. III, 3rd figure in 2nd row).

After 36 hours the trophozoite begins to lose its amœboid shape: the cytoplasmic outgrowths are retracted, the vacuoles tend to disappear and the parasite is transformed into an irregularly rounded and more compact form, representing the schizont. The schizont continues to grow slowly, while its nucleus undergoes repeated divisions (Pl. III, 3rd figure in 3rd row) until from 12 to 24 (average 16) nuclei are present. The schizont, which is now mature, fills the entire enlarged host-cell, only the outer rim of which, containing Schüffner's dots, is visible around the parasite (Pl. III, 3rd figure in 4th row). The pigment granules, which have been scattered in the trophozoite, begin to collect together in the schizont, finally clumping into one or two masses as it approaches maturity.

After the nuclei have ceased to divide the schizont undergoes segmentation, giving rise to as many merozoites as the number of nuclei present (about 16) and leaving the pigment behind. Segmentation is followed by rupture of the host-cell and liberation into the blood of the merozoites and pigment. The merozoites, which are rounded bodies measuring about  $1.5\mu$ , enter new erythrocytes in which the asexual cycle is repeated. The process of schizogony takes about 12 hours, while the entire asexual cycle occupies 48 hours. Though most of the parasites attain maturity at the same time, the development of some of them may be retarded or advanced, with the result that at any given time the majority will be represented by the same stage, while a minority might be in other stages of development. The degree of infection in Benign Tertian malaria is much lower than in Malignant Tertian, the density of parasites rarely exceeding 50,000 per  $\mu$ l. (= c.mm.) of blood, while multiple infection of the erythrocyte is rarely encountered.

The gametocytes may be present in the blood within 5 days after the first appearance of the asexual parasites. As a rule the development from merozoite to gametocyte, which usually takes place in the blood vessels of the spleen and bone-marrow, occupies about 4 days. In the course of this development the parasite remains compact (Fig. 32, f) without producing a vacuole or amœboid outgrowths which characterize the trophozoites. The effect upon the host-cell is the same as in the older trophozoites, i.e. the erythrocyte is enlarged, decolorized and stippled with Schüffner's dots. Both the macrogametocyte and microgametocyte (Pl. III, 3rd figure in 5th and 6th rows), which occur in the peripheral blood, are large round or oval bodies filling practically the entire enlarged erythrocyte which may be invisible or reduced to a thin stippled membrane surrounding the parasite. The nucleus is usually situated near the periphery of the body in which numerous pigment granules are irregularly scattered. The female gametocyte has dense cytoplasm staining dark blue and a small compact nucleus, while the cytoplasm of the male gametocyte is either pale blue or reddish in colour, and the nucleus is large and diffuse. Here it may be noted that schizonts are sometimes mistaken for gametocytes but this confusion should not arise, if it is borne in mind that the body of the latter has a regular circular or oval outline, while the schizonts may be irregular or amœboid in shape. Moreover, the pigment is scattered in the

cytoplasm of the gametocytes instead of being concentrated in one or two masses. Finally, the gametocytes are invariably uninucleate, whereas even the young schizonts may show signs of nuclear division.

The sexual cycle of *P. vivax* in the mosquito has already been described above (Fig. 32). Its duration varies with the external temperature, lasting 16 days at 20° C. and 17–18 days between 23° C. and 28° C. In young oocysts there are numerous (30–40) light brown pigment granules scattered over the body without a definite pattern.

#### xxxiv. *PLASMODIUM MALARIAE* (GRASSI & FELETTI, 1890)

*Plasmodium malariae* is responsible for the Quartan type of malaria which runs a chronic course with relapses after prolonged periods of time. Quartan malaria is a relatively rare disease and accounts for only about 7 per cent. of all cases in the world. It is more common in countries with a temperate climate than in the tropics and occurs in isolated foci, generally within the period from July to September.

The periodicity of the asexual cycle of development is of the quartan type, lasting 72 hours, and the course of development of the parasite is usually well synchronized.

Nothing is known regarding the earliest stages of development of *P. malariae* after inoculation of the sporozoites but it is probable that—as in the case of *P. falciparum* and *P. vivax*—this development occurs outside the blood stream. Furthermore, the existence of secondary exoerythrocytic stages developing side by side with the erythrocytic stages can be inferred from the fact that Quartan malaria shows a strong tendency to relapse.

As in the case of *P. vivax*, the entire asexual life-cycle of *P. malariae* takes place in the blood. The young ring-forms (Pl. III, 2nd figure in 1st row) are somewhat similar to those of *P. vivax*, in that their diameter is equal to about one-third of that of the normal erythrocyte, but the circle of cytoplasm is slightly broader and tends to stain darker blue. However, it is practically impossible to distinguish between the young trophozoites of the quartan and tertian parasites. In accordance with the longer period of asexual development, growth of *P. malariae* is slower than in other species.

After a few hours the vacuole of the ring-form disappears and the trophozoite assumes a compact form, without producing pseudopodial outgrowths of the cytoplasm which are characteristic of the corresponding stage in *P. vivax*. By the end of 24 hours the trophozoite of *P. malariae*, if rounded, has a diameter of half of that of the host-cell, and continues to grow slowly for the next 30 hours, the entire development of the trophozoite thus occupying about 54 hours. The trophozoites may be rounded or elongated. In the latter case they stretch right across the host-cell and are then known as *band-forms*, which are very common in *P. malariae* and represent a characteristic feature of this species (Pl. III, 2nd figure in 2nd row). Coarse dark pigment appears quite early in the trophozoites, the granules being scattered through the cytoplasm.

During the succeeding 18 hours the parasite multiplies by schizogony, in the course of which the nucleus of the schizont divides repeatedly, giving rise to from 6 to 10 daughter-nuclei (average 8), and finally the body is segmented into a corresponding number of merozoites. Two of these stages, representing an immature and a mature schizont are shown in Pl. III (2nd figure in 3rd and 4th rows respectively). The daughter-elements (nuclei and merozoites) of the mature schizont, or segmenter, which occupies almost the entire host-cell, form an irregular cluster, or they may be arranged symmetrically around the centre in the form of a daisy or rosette ; hence the name *rosette-forms* for such schizonts. In young schizonts the pigment granules are scattered in several groups but in the mature schizont they typically form a single mass.

*P. malariae* produces practically no changes in the infected red blood corpuscles. Their size usually remains unaltered, though sometimes they appear to be even smaller than the normal cells, and as a rule they are not stippled, though occasionally after intensive staining the host-cell may reveal a few pink dots, or *Ziemann's* stippling.

The degree of infection in Quartan malaria is lower than in any of the other types of the disease, the density of parasites rarely exceeding 10,000 per 1  $\mu$ l. (= c.mm.) of the blood.

As in the other species of malaria parasites, the gametocytes of *P. malariae* develop from merozoites in the inner organs and usually appear in the blood stream when fully grown. The mature gametocytes (Pl. III, 2nd figure in 5th and 6th rows) are round or oval and

fill the entire red blood corpuscle, which retains its normal size. Except for their size, the gametocytes of *P. malariae* closely resemble those of *P. vivax* and can be described as replicas of the latter in miniature. The macrogametocyte stains deep blue and its nucleus is small, while the microgametocyte is pale blue or reddish and has a diffuse large nucleus. In both sexes the nucleus is situated on the periphery of the body and the pigment granules are irregularly dispersed in the cytoplasm. The latter feature serves to distinguish the gametocytes from immature schizonts of *P. malariae*, in which the pigment is clumped into a single mass. As a rule, gametocytes of this species occur in the blood in scanty numbers.

The sexual cycle in the mosquito is similar to that already described. The development of this parasite in the vector is slower than in the other species. At 20° C. it takes 30-35 days, while between 23° C. and 28° C. it takes 28 days. In young oocysts the large dark pigment granules are concentrated in one clump.

#### xxxv. *PLASMODIUM OVALE* STEPHENS, 1922

*Plasmodium ovale* has only recently been recognized as a distinct species and is still rarely encountered. It causes a tertian type of malaria (Ovale Tertian), similar to but milder than Benign Tertian, the periodicity of its asexual cycle being 48 hours.

Ovale Tertian malaria has been recorded chiefly from tropical Africa, but recently isolated cases have also been reported from New Guinea, South America and south-eastern Russia.

*P. ovale* has many features in common with both *P. malariae* and *P. vivax*. Thus, it resembles the former species morphologically, while its effect upon the host-cell is somewhat similar to that produced by the latter. The entire asexual cycle of *P. ovale*, lasting 48 hours, takes place in the peripheral blood. The young ring-forms (Pl. III, 4th figure in 1st row) occupy about one-third of the diameter of the host-cell which at this stage is of normal size. The circle of cytoplasm usually stains dark blue, as in *P. malariae*, but on the whole there is little to distinguish the ring-forms of *P. ovale* from those of *P. vivax*. However, in infections with *P. ovale* the red cells harbouring ring-forms acquire a heavy stippling with Schüffner's dots which may be present in up to 100 per cent. of the host-cells, whereas in the case of *P. vivax* the stippling only appears in older

trophozoites. This early stippling is the main differential character distinguishing *P. ovale* from *P. vivax* in the ring stage. As the trophozoites grow they continue to show a resemblance to *P. malariae* in their solid appearance and absence of amœboid outgrowths. However, they differ from the trophozoites of *P. malariae* in the rare occurrence of band-forms, most of the older trophozoites of *P. ovale* being represented by compact round forms which contain a few granules of dark pigment. At this stage a large proportion of the infected red cells are slightly enlarged, many of them assuming an oval shape with or without ragged or fimbriated margins, while Schüffner's dots are plentiful and appear to extend almost beyond the edges of the red cell (Pl. III, 4th figure in 2nd row). The schizonts are also like those of *P. malariae*, i.e. they are round compact bodies not exceeding the normal erythrocyte in size but the host-cells are usually slightly enlarged and frequently (about 25 per cent.) oval in shape, with well-defined stippling (Pl. III, 4th figure in 3rd row). The mature schizont usually contains 8 nuclei which are irregularly arranged round a central clump of pigment granules, without giving rise to a symmetrical rosetta-form (Pl. III, 4th figure in 4th row).

The gametocytes of *P. ovale* are also similar to those of *P. malariae* both as regards size and appearance but the red blood corpuscles harbouring them contain Schüffner's dots and may be slightly enlarged, though they seldom assume the oval shape characteristic of the host-cell in the schizont stage. The scanty pigment granules in the sexual forms are scattered through the cytoplasm (Pl. III, 4th figure in 5th and 6th rows). As in the other species, the macrogametocyte stains dark blue and has a small nucleus, whereas the microgametocyte is pale blue or reddish and has a large diffuse nucleus.

The oval shape and ragged margins of the red blood corpuscles harbouring the later stages of development of *P. ovale* are characteristic of this species and therefore of diagnostic value. However, these features are not revealed in fresh blood but are evidently produced in the course of making the blood film and indicate some mechanical weakness on the part of the parasitized erythrocytes.

In general, *P. ovale* can be characterized as follows: (a) it is morphologically similar to *P. malariae* in all its stages, but (b) the periodicity of its asexual cycle is like that in *P. vivax*; (c) the changes which *P. ovale* produces in the host-cell are somewhat similar to

(c) Schizonts.—The schizonts of *P. vivax* lie in considerably enlarged and stippled red blood corpuscles and are themselves larger than a normal erythrocyte. Those of *P. ovale* are smaller than the slightly enlarged and stippled host-cell, which is often oval and/or fimbriated. The schizonts of *P. malariae* are small and almost fill the unaltered red cell. Schizonts of *P. falciparum* are not usually encountered in films of the peripheral blood but, when present, they can be recognized by their small size, which does not exceed two-thirds of the diameter of the unaltered host-cell. In addition to the features mentioned above, the mature schizonts, or segmenters, of the four species of malaria parasites can be differentiated by the number of nuclei or merozoites present.

(d) Gametocytes.—The crescent-shaped gametocytes of *P. falciparum* cannot be mistaken for those of any other species. The rounded sexual forms of *P. vivax* are much larger than a normal erythrocyte, while those of *P. ovale* are not larger than a normal red cell but only partly fill the slightly enlarged and stippled host-cell. The gametocytes of *P. malariae* are small, filling the entire unaltered host-cell.

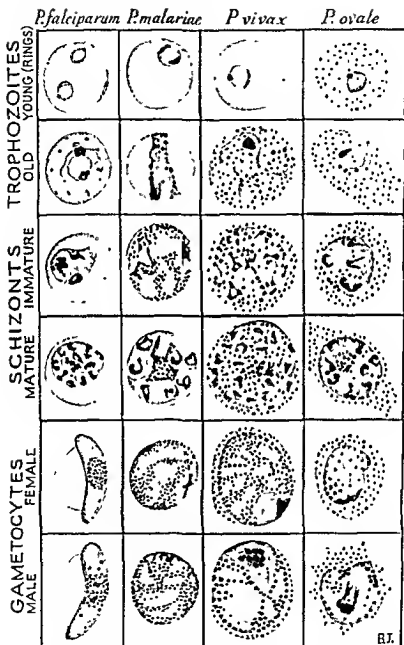
(e) Pigment.—In *P. vivax* the pigment granules are fine and light brown in colour; in *P. malariae* they are coarse and dark brown; in *P. falciparum* and *P. ovale* they are coarse and blackish brown. In all four species the pigment is usually scattered in all the stages except the schizonts, in which the granules are clumped together in one or two masses. However, in *P. falciparum* it begins to form clumps in the growing trophozoite.

### HOST-PARASITE RELATIONSHIP

Malarial infection is characterized by a series of clinical attacks alternating with intervals of quiescence, or latent periods. The clinical manifestations following the inoculation of sporozoites represent the primary attack, while the subsequent attacks are known as relapses. The duration of the primary attack, the length of the latent periods, as well as the liability to relapse vary with the species of *Plasmodium*.

The Febrile Paroxysm.—An attack is manifested by periodic paroxysms of acute fever which are closely correlated with the asexual cycle of development of the parasite in the blood (Fig. 34). During the early stages, while the trophozoites are growing, and

# PLATE III.



THE MALARIA PARASITES: DEVELOPMENTAL STAGES IN MAN.  
(X 2000).

(Adapted from various authors)

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later, when the schizonts are undergoing nuclear division, the patient's temperature remains normal. However, as soon as segmentation is completed and the infected red blood corpuscles are ruptured, releasing the merozoites into the blood stream, the paroxysm of fever is produced, its development proceeding in three phases: (a) the cold or shivering phase ("rigor"), lasting up to 1-2 hours, in the course of which the patient's temperature rises rapidly; (b) the hot phase, lasting several hours, when the temperature reaches its peak and may exceed  $40.5^{\circ}\text{C}$ . ( $105^{\circ}\text{F}$ .); and (c) the sweating phase, lasting 2-5 hours, when the temperature rapidly falls to the normal level. The duration of the entire febrile paroxysm may vary from a few hours to a day or longer. During the decline of fever the merozoites enter new red blood corpuscles, thus initiating the asexual development of another generation of parasites which is completed during the succeeding afebrile interval.

The paroxysms are repeated on subsequent days as each generation of the parasite attains maturity, the periodicity of fever in the different types of malaria corresponding to the time the parasites take to complete schizogony. This is 48 hours in the case of *P. falciparum*, *P. vivax* and *P. ovale*, and 72 hours in *P. malariae*; therefore theoretically the periodicity of symptoms is tertian in Malignant, Benign and *Ovale* Tertian infections, and quartan in Quartan malaria. However, as a rule, at the onset of an attack the development of the parasites is not closely synchronized, since broods belonging to the same generation mature at different times, with the result that the febrile symptoms assume an irregular character. Thus two broods of *P. vivax* maturing on alternate days, or three broods of *P. malariae* maturing at intervals of 24 hours, will produce daily paroxysms, or *quotidian* periodicity, instead of tertian and quartan periodicity characteristic of these species respectively, when the development of each generation of parasites is synchronized. Later in the course of the primary attack the periodicity of symptoms may become typical, probably owing to the suppression of superfluous broods. It also tends to be typical in relapses.

The febrile reaction thus coincides with the release into the circulation of merozoites together with pigment and debris of red cells. Its exact nature is not clearly understood but it is thought

that it represents a reaction to the introduction of foreign protein into the blood.

Here it may be noted that the terms "tertian" and "quartan," denoting the periodicity of fever, are based on the Roman method of reckoning, according to which the day on which an event (e.g. fever) takes place is known as the first day, while the second and third days on which the event recurs are described as the third (tertian) and fourth (quartan) days respectively.

**Pyrogenic Level**—The clinical symptoms manifested by a paroxysm of fever are not produced when only small numbers of parasites are present. It has been estimated that the minimum number of parasites capable of producing fever is 1 parasite per 100,000 red blood corpuscles, or 50 parasites per 1  $\mu$ l. (= c.mm.) of blood, which is equivalent to 150 million parasites in a man weighing 10 stone (64 kg.). This density is known as the THRESHOLD or PYROGENIC LEVEL.

**Incubation Period.**—The time required by the parasites to reach this concentration in the blood and to provoke the first clinical symptoms, manifested by a rise of temperature to 37.8° C. (100° F.), represents the incubation period. The length of the incubation period depends, in the first place, on the number of sporozoites inoculated by the mosquito-vector and, secondly, on the species and strain of malaria parasite. The relevant facts regarding the influence of the number of sporozoites are as follows. After invading the salivary glands of the mosquito the sporozoites are found both within the secretory cells and in the ducts. After the mosquito has had a number of blood meals the supply of sporozoites in the ducts is gradually exhausted but the cells continue to harbour them. While the ducts are heavily infected the mosquito injects into man numerous sporozoites and the resulting infection has a short incubation period, but in subsequent bites, when the ducts are free of sporozoites, the inoculum contains only scanty numbers of sporozoites derived from the cells. In such cases the incubation period may be prolonged for months. There is reason to believe that in the case of *vivax* malaria about 2,000 sporozoites are necessary to produce an infection with a normal incubation period.

The average incubation period for *P. falciparum* is 11 days, for *P. vivax* and *P. ovale* 15 days, and for *P. malariae* 28 days. The longer incubation period in Quartan malaria is probably due to the fact that each schizont produces only about 8 merozoites every 72

hours, whereas *P. vivax* produces about 16 merozoites every 48 hours.

In general, therefore, the length of the incubation period in malaria varies inversely with the number of sporozoites introduced by the mosquito and corresponds to the rate of asexual multiplication of the parasite in the human host.

### Course of Infection

The course and duration of infection varies according to the type of malaria. As a rule, the primary attack is succeeded by a latent period during which the patient presents no febrile symptoms though harbouring parasites; subsequently there may be relapses alternating with afebrile intervals over a variable period. The tendency to relapse is most marked in *P. malariae* and least in *P. falciparum*. In general, the course of malarial infection may be said to comprise an acute phase, represented by the primary attack, and a chronic phase which covers the period from the end of the primary attack to recovery, during which the patient becomes a parasite-carrier, showing no symptoms in the latent intervals between relapses. The probable mechanism of malaria relapses will be discussed below in connexion with immunity.

We can now consider the characteristic features of the four types of malaria.

**Malignant Tertian.**—This is the most dangerous form of malaria, especially during the primary attack, and may end fatally unless promptly treated. Its normal incubation period is 8–14 days. The development of *P. falciparum* is less synchronized than that of the other species and the periodicity of fever is consequently more irregular in this type of malaria. There are frequently several broods of parasites, the segmentation of which is separated by varying intervals. When two broods are present and the interval is 24 hours, the periodicity is quotidian, but when this interval is shorter there may be two febrile paroxysms on the same day. There may even be three or more broods developing independently, each causing its own paroxysm. As a result fever may be irregular and prolonged. After the termination of the primary attack, which may last 10–14 days, relapses with milder symptoms may occur in quick succession over a period usually not longer than 2 months, but some cases may relapse up to 6–9 months. Generally the duration of *P. falciparum*

infection does not exceed one year. It is a characteristic feature of *falciparum* malaria that parasites are not readily detected in the peripheral blood at the height of the febrile paroxysm but are most numerous a few hours later, after the temperature has fallen.

The greater severity of Malignant Tertian as compared with the other types of malaria is due, in the first place, to the higher rate of multiplication of the parasite, which rapidly increases in numbers during the infection; secondly, to the fact that schizogony takes place in the capillaries of the inner organs which become blocked, owing to agglutination of the infected red cells and their retention in these vessels; furthermore, the localization of schizonts in certain organs accounts for the multiplicity of symptoms; thirdly, to lack of synchronization of the asexual cycle of development which results in irregular and protracted fever.

Benign Tertian.—Though the normal incubation period of this type of malaria is between 9 and 15 days, it may be considerably prolonged, the infection remaining latent for periods up to one year. This latency is probably the result of the injection of an insufficient number of sporozoites by the mosquito (see above). However, some observers believe that the length of the incubation period of *P. vivax* may differ in various geographical strains, northern ones having a long incubation period and southern ones a short one. As in the case of *falciparum* malaria, in the early phases of the primary attack the development of the parasites is not well synchronized. There are commonly two broods undergoing segmentation on alternate days and producing a quotidian periodicity of fever for several days. However, later the asexual cycle becomes synchronized and the paroxysms assume the typical tertian periodicity. Benign Tertian malaria shows a greater tendency to relapse than the Malignant Tertian disease. When the primary attack—which may last from 2 to 4 weeks—subsides the patient may remain free from fever for several weeks, after which he is liable to have a succession of relapses with increasingly longer latent intervals between them. The duration of *vivax* infection does not as a rule exceed 3 years. In the initial phase of the primary attack parasites are scanty (sometimes less than 10 per  $\mu$ l. of blood) but later their number increases considerably. As long as the periodicity of fever remains quotidian parasites of every stage of development may be present in the blood at the same time, but when it assumes a tertian

periodicity the majority of parasites found at any given time belong to the same age group. The parasites also persist in the blood in appreciable numbers during the latent periods of the infection intervening between the relapses, finally disappearing after immunity has been established.

**Ovale Tertian.**—Malaria due to *P. ovale* produces the mildest symptoms. It may terminate spontaneously after the primary attack, which lasts a few days, and as a rule does not produce relapses. The paroxysms are usually of the typical tertian character and parasites are present in the blood during both the febrile and afebrile periods. Owing to the resemblance of *P. ovale* and *P. vivax*, an *ovale* infection may be mistaken for Benign Tertian malaria but from the clinical point of view the error of diagnosis is not detrimental to the patient.

**Quartan.**—The evolution and course of Quartan malaria are slower than in the other types of the disease. The incubation period usually extends to one month, while the primary attack may last for one or two months. The paroxysms of fever, which are mild and short (4–6 hours), may at first have a quotidian periodicity but later they assume a quartan character. Parasites are present in the blood during both the febrile and afebrile periods but their number is always relatively small. *P. malariae* has a marked tendency to produce relapses which may occur several years—in some cases up to 20 years—after the original infection was contracted. There is reason to believe that during the latent period parasites persist in small numbers in the blood: this accounts for the ease with which Quartan malaria is transmitted in the course of blood transfusion from donors harbouring *P. malariae* for some years.

**Mixed Infections.**—Occasionally malarial infection is caused by more than one species of *Plasmodium*. In such mixed infections there is sometimes an antagonism or incompatibility between the different species, on account of which only one of them may be present in the blood at any given time. Thus in a mixed infection of *P. falciparum* and *P. vivax*—which is the most common combination—the latter is first suppressed and the primary attack is due to *P. falciparum* but relapses occurring subsequently are produced by *P. vivax*. If *P. malariae* is present, it persists longer than either of the other two species.

**Strains of Parasites.**—Each of the four species of malaria para-

malaria is longer than in that induced by blood inoculation, usually occupying 10–20 days. The retardation is probably due to the pre-erythrocytic development initiated by the sporozoites.

**Accidental Malaria.**—Malaria can be inoculated accidentally in the course of blood transfusion from donors harbouring a latent infection. Infections contracted in this way are especially common in the case of *P. malariae* and have been reported many years after the donor had left an endemic area. Such infections have also been

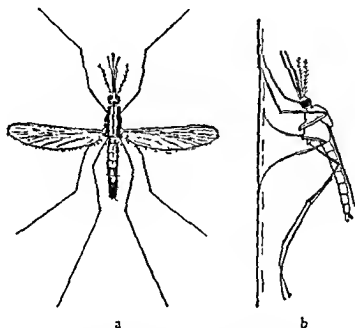


FIG. 37.—FEMALE *Anopheles*: VECTOR OF MALARIA. (After Edwards and James, 1943 : *British Mosquitos and Their Control*; and Wenyon, 1926.)  
Dorsal (a) and side (b) views.

known to occur following the transfusion of blood stored at 0° C. for a fortnight. There have also been numerous cases of transmission of malaria among drug addicts using the same hypodermic syringe.

**Congenital Malaria.**—There are a fair number of records of malarial infection in new-born infants which has undoubtedly been acquired from an infected mother before birth. Normally the barrier between the maternal and foetal circulation is extremely efficient, even when the placenta is heavily parasitized, and it is thought that congenital malaria may be acquired either as the result of damage to the placenta or through an abrasion at birth.

## Cyclical Transmission

The human malaria parasites are incapable of developing in any mosquitos except those belonging to the genus *Anopheles*. The infection of mosquitos in a locality depends upon the incidence of infection among the population but as a rule the proportion of naturally infected *Anopheles* is well below 10 per cent. Though various species of anopheline mosquitos may be present in an endemic area, only some of them are responsible for the spread of malaria. The most important vectors in different countries are shown in Table 13.

TABLE 13  
CHIEF VECTORS OF MALARIA

COUNTRIES	SPECIES OF <i>Anopheles</i>
Netherlands, Spain, Portugal . . . . .	<i>A. maculipennis atroparvus</i>
Spain, Italy, Dalmatian coast, Sicily, Sardinia, Corsica, N.W. coast of Africa . . . . .	<i>A.m. labranchiae</i>
Rumania . . . . .	<i>A.m. messeae</i>
Southern Europe, Middle East . . . . .	<i>A. superpictus</i>
Mediterranean, Arabia, Iraq, Iran, India, Burma . . . . .	<i>A. stephensi</i>
Balkans, Palestine, Near East . . . . .	<i>A. sacharovi</i> (= <i>elutus</i> )
Palestine, Syria . . . . .	<i>A. claviger</i> (= <i>bifurcatus</i> )
India, Burma, Malaya, Indo-China, Netherlands Indies . . . . .	<i>A. sundaicus</i> (= <i>ludlowi</i> )
North India, Burma, Indo-China, South China . . . . .	<i>A. minimus</i>
Malaya, Netherlands Indies . . . . .	<i>A. maculatus</i>
India, Burma, Malaya, Siam, Indo-China	<i>A. fluviatilis</i> (= <i>listoni</i> )
Ceylon, India . . . . .	<i>A. culicifacies</i>
West Africa . . . . .	<i>A. melas</i>
Egypt, Arabia, Tropical Africa . . . . .	<i>A. gambiae</i> (= <i>costalis</i> )
Tropical Africa . . . . .	<i>A. funestus</i>
United States of America . . . . .	<i>A. quadrimaculatus</i>
Central America, Andes, Brazil, Vene- zuela, Argentina . . . . .	<i>A. darlingi</i>
Central America, Caribbean area . . . . .	<i>A. albimanus</i>
Panama, West Indies, North Brazil . . . . .	<i>A. aquasalis</i>
Trinidad . . . . .	<i>A. bellator</i>
Polynesian and Australian regions. . . . .	<i>A. punctulatus</i>



## Immunity

Although human beings vary in their susceptibility to infection with the malaria parasites, there is no evidence of complete resistance, or absolute *natural immunity*, to infection. However, in hyper-endemic areas of malaria the native population may possess a high degree of *natural tolerance* to infection with local strains of parasites. In such persons parasites are usually present in small numbers without producing clinical symptoms. A certain degree of *racial immunity* may be present in human races who have been exposed to infection for many generations. Thus, American Negroes show a natural tolerance to infection with *P. vivax*, which produces only mild symptoms in them. On this account it is usual to employ *P. falciparum* in malaria-therapy of Negro paretics.

There is also considerable evidence of *acquired immunity* in malaria. Thus, if the infection is allowed to run its natural course, the acute primary attack may terminate in recovery (as in *ovale* infection) or it may be followed by a latent period with a marked diminution in the number of parasites. As a rule, the parasites again increase in numbers from time to time and give rise to relapses, which alternate with latent intervals until the patient recovers. The spontaneous restriction of the number of parasites is also evident from the following data. On theoretical grounds one might expect each schizont of *P. vivax* to produce 24 merozoites every 48 hours, with the result that after 14 days the number of parasites descended from a single schizont would be somewhere between 200 and 300 million. If this rate of multiplication continued unchecked the parasites would soon overrun and kill the host. Actually, however, the density of *P. vivax* does not usually exceed 50,000 per  $\mu$ l. of blood and that of *P. malarie* is seldom higher than 10,000 per  $\mu$ l., whereas in *P. falciparum* it may be more than 500,000 per  $\mu$ l. Furthermore, the mortality rate in malaria is relatively low, as a rule not exceeding 2 per cent. of the persons suffering from this disease.

Recent studies of malaria in man, monkeys and birds have thrown much light on the nature and mechanism of immunity in this disease. The available data suggest that acquired immunity involves cellular factors (phagocytosis) and humoral factors (antibodies), both of which are intimately connected with the reticulo-endothelial system.

In the initial stages of malarial infection the multiplication and pathogenic effects of the parasite are controlled by phagocytosis which at this stage is non-specific and represents a *natural* defence mechanism of the host. As the attack develops the reticulo-endothelial system is stimulated to further activity which is reflected in local hypertrophy (splenomegaly) and in considerable increase of the number of macrophages. In most cases this non-specific phagocytosis is sufficient to check excessive multiplication of the parasites but in others (e.g. in pernicious cases of *falciparum* malaria) it may fail to do so and the patient dies during the acute attack. However, if he survives, the slow non-specific activity of the macrophages is replaced by an active *specific* phagocytosis of the parasites, resulting in a marked diminution of their numbers and in the cessation of clinical symptoms. Evidence of phagocytosis is provided by the presence in the reticulo-endothelial cells of the spleen and other organs both of engulfed parasites in various stages of digestion and of deposits of malaria pigment. Pigment may also occur in leucocytes circulating in the blood, thus supplying an additional clue to malarial infection. There is reason to believe that, in addition to phagocytes which are chiefly concerned with the destruction of the parasites, the defence mechanism also includes antihodies which neutralize the toxins released by the parasites. As the result of immunity acquired in the initial stages of the disease there is a tendency for the infection to become latent for varying periods of time during which the number of parasites is kept at a low level by the specific defence mechanism of the host, while clinical symptoms are either absent or may recur from time to time. It is thought that such relapses may be due to the persistence in the host of exoerythrocytic forms which are not affected by the immune mechanism and serve to repopulate the blood with a sufficient number of parasites to bring about a clinical attack.

The immunity acquired in the course of malaria combines features of concomitant immunity, or premunition, with those of residual immunity (see Chapter 3). Under natural conditions its maintenance depends on the exposure of the host to repeated infections spread over several years, in the course of which the host may remain a parasite-carrier showing no symptoms of disease. But while in typical premunition the host loses any resistance to reinfection which he may have acquired, if the infection disappears (spon-

taneously or as a result of treatment), in malaria premunition is followed by a residual immunity, which may last for several years. Immunity in malaria is also characterized by its strain-specificity as well as species-specificity. It has already been noted that each of the species of human malaria parasites is made up of a number of distinct strains or races which differ in various biological characters (see Chapter 3), including immunological properties. The immunity acquired in the course of malaria effectively protects the host only against reinfection or superinfection with parasites of a *homologous* strain, i.e. belonging to the same strain as that which caused the original infection. However, the host remains fully susceptible to infection with other strains of the same species (*heterologous* strains) and with different species of *Plasmodium*, both of which will produce clinical symptoms, though in the former case these may be milder than in the primary attack due to the original infection.

The effect of premunition is observed in its purest form in hyperendemic areas, especially in tropical and subtropical countries. In such countries the incidence of malaria is very high (up to 100 per cent.) and the mortality among children under the age of five is considerable. Those who recover acquire premunition which is built up and maintained by repeated infections. In view of the strain-specificity of premunition complete protection against malaria can only be acquired if the children are immunized against all the local strains, each of which must run its normal course of infection. This process might require up to 15 years, during which the individual is exposed to almost continuous reinfections with malaria parasites. From the age of fifteen upwards natives of highly endemic areas are usually fully premunized: they do not manifest any clinical symptoms but may remain carriers, the density of parasites in their blood frequently not exceeding one per microscopic field. In localities of lower endemicity, where exposure to infection is not continuous throughout the year, the process of immunization is delayed and incomplete, with the result that adults as well as children suffer from febrile attacks.

Drug treatment may interfere with the development of premunition if a cure is effected in the initial phase of the disease, but if treatment is only sufficient to check the attack without eradicating the infection, a certain degree of immunity will develop. Evidently a large amount of antigen liberated by the parasites over a short

period of time is less effective in stimulating the defence mechanism of the host than the same amount spread over a longer period.

The rigid strain-specificity of immunity accounts for the occurrence of outbreaks of malaria within endemic areas, which may be caused by the immigration from other localities of persons harbouring different strains of malaria parasites. It is also one of the reasons of failure to produce a vaccine against malaria, since such a vaccine would have to be extremely polyvalent in order to afford protection against various strains of malaria parasites which are likely to be encountered.

### Simian Malaria Parasites

In addition to man, species of *Plasmodium* occur in various other Primates, including the anthropoid apes and various monkeys of the Old and New World. Some of the simian parasites closely resemble the human malaria parasites and a few are transmissible to man.

The anthropoid apes—chimpanzee and gorilla—harbour three kinds of malaria parasites which are morphologically indistinguishable from the three species parasitic in man: *P. falciparum*, *P. vivax* and *P. malariae*. In order to determine the mutual relationships between the human and anthropoid parasites, cross-infection experiments have been carried out. In the case of the *falciparum*-like parasites attempts to infect man from chimpanzee and *vice versa* have so far failed. As regards the *vivax*-like parasite, the human strain when introduced into the chimpanzee produced in the latter an inapparent infection which could only be demonstrated by subsequent infection of another human being from the ape; on the other hand, man appears to be more refractory to the ape-strain, which may produce in him a transient infection. Man proved to be most susceptible to infection with the *malariae*-like parasite of the chimpanzee which provoked in the human host febrile symptoms of typical quartan malaria and was carried through several human passages; however, in chimpanzees infection with the human strain was symptomless, with a few parasites.

Some authors maintain that, because of the high degree of host-restriction, the human and anthropoid malaria parasites should be regarded as distinct species. The parasites of the apes were accordingly given the following names: *P. reichenowi* for the one

corresponding to *P. falciparum*; *P. schwetzi* for the one like *P. vivax*; and *P. rodhaini* for the one like *P. malariae*. However, in view of the absence of any morphological differences between them, it is obvious that the human and anthropoid parasites are limited to three species, each comprising two biological races, one adapted to man, the other to apes, while the specific names to be borne by the malaria parasites of these primates should be those applied to the corresponding human forms.

Among the malaria parasites of monkeys the most important one from the medical point of view is *P. knowlesi* which occurs in Asiatic macaques, especially *Macacus irus*. *P. knowlesi* has a quotidian periodicity, the entire asexual cycle—which takes place in the peripheral blood—lasting 24 hours. The ring-forms (Fig. 36, d) occupy from one-third to half the diameter of the erythrocyte. As they grow the trophozoites (Fig. 36, e) become solid and rounded. The schizonts occupy from two-thirds to almost the whole of the red blood corpuscle, according to age (Fig. 36, f, g), and give rise, to from 8 to 16 (usually 10) merozoites. The infected red cells remain normal in size throughout all stages of development of the parasite. Stippling is usually absent but it may be brought out in some cells by special methods of staining. The only change undergone by the host-cell may be a distortion in those harbouring the older parasites: such cells may become oval (Fig. 36, g) or fimbriated (Fig. 36, e) like the red cells in *ovale* malaria (cf. Pl. III). Pigment is abundant in all stages of development. The gametocytes (Fig. 36, h) of *P. knowlesi* are spherical and fill almost the entire red blood corpuscle.

*P. knowlesi* produces mild symptoms in its natural host, *Macacus irus*, and the infection usually terminates in spontaneous recovery. However, in some other macaques, especially *M. rhesus*, the parasites may reach enormous numbers and the disease runs an acute course, terminating in the death of the host.

*P. knowlesi* is of special interest in medical practice because it is readily transmissible to man and is sometimes employed in malaria-therapy of paretics. The incubation period in man is 4-6 days after intravenous inoculation. The fever, which is quotidian, lasts 10 days, after which the patient recovers spontaneously. One of the advantages of induced monkey malaria in man, in addition to its mild course, is the ease with which the strain can be

maintained in *rhesus* monkeys. The acute phase of the disease in this animal can be controlled with quinine, after which the infection continues to run a mild chronic course.

### DIAGNOSIS

The diagnosis of malaria is not conclusive unless the presence of parasites in the patient can be demonstrated. Although the number of parasites in the blood varies considerably in the course of malarial infection and they may be temporarily undetectable, especially in *falciparum* malaria or during drug treatment, they can usually be discovered at some phase of the disease.

(1) Blood Examination.—The only reliable diagnostic method is the microscopic examination of the patient's blood. In general, blood films should be made and examined when the patient first comes under observation and subsequently as the occasion demands, but it should be borne in mind that the parasites are more easily detected several hours after the height of the paroxysm has been reached. Since the entire asexual development of *P. vivax*, *P. malariae* and *P. ovale* takes place in the peripheral blood, in Benign Tertian, Quartan and Ovale Tertian infections parasites can be found in the blood during both the afebrile and febrile periods, but in Malignant Tertian malaria the parasites usually disappear from the peripheral blood during the afebrile period to undergo further development in the inner organs, therefore in this case it is advisable to examine the blood a few hours after the febrile paroxysm has reached the peak.

Though malaria parasites can be recognized in fresh unstained preparations of the blood, in practice their detection and identification are based on the examination of blood films stained by one of the Romanovsky methods. The technique of preparation and staining blood films is fully described in Chapter 17.

It is advisable to make both thick and thin films every time the blood is examined. The thick film is essentially a concentration method and is used mainly for the detection of the presence of malaria parasites, the specific identification of which is usually made in the thin film, though some characteristic stages of the different species can also be recognized in the thick film.

In practice it is best to start with the examination of the thin film : if parasites are present, there is no need to examine the thick

film but if they cannot be detected in a few minutes, the thick film is examined. If parasites are discovered in the latter but cannot be identified with certainty, the thin film is re-examined more thoroughly until the nature of the infection can be determined.

In a well stained thin blood film containing a sufficient number of parasites it is usually easy to identify the species of *Plasmodium* present. This can be done with the aid of Table 12 and the summary of differential characters given above, while Pl. III illustrates the most important stages of development. However, it may be difficult to identify the species of parasite from the examination of only a few individuals, since some stages, e.g. ring-forms, may be indistinguishable. A correct diagnosis is the result of cumulative evidence based on a critical inspection of a sufficient number of parasites in different stages of development. The finding of leucocytes with ingested malaria pigment provides an additional clue to infection which, in the absence of detectable parasites, should lead to a renewed search for them.

Although the parasites are readily detected in a thick blood film they may be difficult to recognize and identify. This is due, in the first place, to the fact that the parasites appear in an unfamiliar setting, owing to the obliteration of the red blood corpuscles in the thick film as the result of dehaemoglobinization. The only elements seen in the film are the leucocytes and the parasites. However, the appearance of the latter is altered, owing to lack of fixation and slow drying of the blood in the course of preparation of the film. Thus, the young trophozoites of all species may appear as incomplete rings or streaks of blue cytoplasm with a detached chromatin dot; in old amœboid trophozoites of *P. vivax* the cytoplasm may be fragmented and Schüffner's dots are sometimes not stained, while band-forms of *P. malariae* lose their characteristic shape. On the other hand, the schizonts and gametocytes of these species may almost retain their normal appearance, and the crescents of *P. falciparum* are unmistakable. Pigment granules also undergo little change in thick films. The interpretation of the parasites seen in a thick film, therefore, requires some experience, which the novice can acquire by first studying the normal morphology of various stages of the parasite in a thin blood film and then searching for the corresponding forms in a thick film made from the same patient.

The thick film is a time-saving method which reveals even slight

infections within a short time. It has been estimated that one microscopic field of a thick film is equivalent to about 50 fields of a thin film. As regards the time spent on the examination of a film before it can be declared negative, it is generally reckoned that 3-5 minutes should be devoted to a thick film, while in thin films from 100 to 250 fields (using a  $\frac{1}{2}$  in. objective with oil immersion) should be inspected: this may occupy from 15 to 30 minutes, according to the experience of the observer. The timing for thin films is based on the following considerations: (a) that in non-immune persons an attack of clinical malaria is associated with the presence of at least one parasite per 100 fields; and (b) that fever can be produced by an infection having a density of one parasite per 100,000 red blood cells (pyrogenic level), the time required to scan this number being about 30 minutes. However, some authorities maintain that if a careful search of a thin film by a competent worker does not reveal any parasites in the course of 5 minutes, it is doubtful if clinical malaria is present. If parasites are not detected in thick and thin films and there is reason to suspect malaria, or if there is any doubt about the diagnosis, fresh blood films should be examined at intervals of several hours for some days. Repeated examinations are of special importance in Malignant Tertian malaria, since in this disease the parasites are sometimes so scanty that they are easily missed. Blood films should be made even if they cannot be examined immediately. In examining thin films it should be remembered that parasites are more numerous along the margins.

(2) *Provocation*.—In cases where there is a strong suspicion of malarial infection but parasites cannot be detected in the blood, an attack is sometimes provoked by injecting into the patient subcutaneously 0.5 ml. of 1:1000 solution of adrenalin 20 minutes before the blood is taken. This causes contraction of the spleen and the emergence of infected red blood corpuscles into the blood stream.

(3) *Sternal Puncture*.—Some workers have recently recommended the examination of smears of bone-marrow obtained by sternal puncture (see Chapter 17) as a supplementary diagnostic method in malaria when parasites cannot be found in the blood. However, it is doubtful if this method has any advantage over the examination of a thick blood film.

(4) *Post-Mortem Examination*.—If death from malaria is sus-





## SECTION C

### PARASITES OF DOUBTFUL NATURE

AMONG the parasites occurring in man there are two groups of unicellular organisms, known as TOXOPLASMS and SARCOSPORIDIA, the exact nature and systematic position of which have not yet been determined. While the Sarcosporidia are primarily parasites of the muscles, the Toxoplasms occur in the blood and the reticulo-endothelial system, on account of which the last-named organisms are also included among the protozoa inhabiting these tissues (Table 5).

## CHAPTER 13

### THE TOXOPLASMS

*DURING* the last quarter of a century evidence has been accumulating of the occurrence in man of peculiar unicellular parasites, which have acquired considerable importance in medicine since the recent discovery of their ætiological rôle in the causation of a distinct disease, now known as *HUMAN TOXOPLASMOSIS*.

The occurrence of the parasites in question among various lower animals (mammals, birds and reptiles) in different parts of the world had been known long before they were found in man. These parasites belong to the genus *Toxoplasma* and are thought to be related to the Sporozoa, though their true affinities are unknown and even their animal nature has not been definitely established.

The toxoplasms found in different animal hosts, including man, are morphologically indistinguishable. Their body (Fig. 38, a-e) has the shape of a small crescent which measures  $3.3-6.5\mu$  in length and  $1.0-3.5\mu$  in breadth, the average dimensions being about  $4 \times 1\mu$ . The ends of the body are unequally pointed, one end usually being blunter and broader than the other. The single nucleus, which is situated near the blunt end of the body, has a vesicular structure and contains a central karyosome but in preparations stained by the Romanovsky methods it has a granular appearance. Near the nucleus there may be a darkly staining granule of unknown function, the so-called "paranuclear body" (Fig. 38, a). There are no organs of locomotion, the parasites being apparently motionless. The toxoplasms are almost invariably intracellular (Fig. 38, f). They are found within reticulo-endothelial cells of different organs and tissues and occasionally in mononuclear leucocytes of the blood, as well as in muscle fibres. They are rarely encountered outside the host-cell but in smears they may be seen free, owing to rupture of the host-cell.

The parasites reproduce by longitudinal binary fission (Fig. 38, d, e). As they increase in numbers they gradually fill and distend the host-cell until the latter becomes unrecognizable, being reduced to a membrane which encloses the parasites. The remains of the host-cell nucleus are sometimes seen adhering to the membrane.

These aggregations, which are known as "pseudocysts," owing to their cyst-like appearance, are usually rounded, measuring from  $5\mu$  to  $50\mu$  in diameter and containing from about half a dozen to more than a hundred parasites. In such pseudocysts the outlines of individual parasites are not always distinctly visible, especially in smear preparations, and they may appear as a single multinucleate mass. This picture has given rise to the erroneous belief that toxoplasms may multiply by schizogony.

The toxoplasms recorded from different animals, including mammals, birds and reptiles, have been given distinct names, mostly after their hosts. Thus the parasite originally discovered in an African rodent, *gondi*, is known as *Toxoplasma gondi*; that found in rabbits is known as *T. cuniculi*; the mouse parasite is *T. musculi*; that of pigeons *T. columbae*, while the one occurring in man bears the name *T. hominis*. However, all these species are not only morphologically identical but the parasites from one host are readily inoculable to a variety of other hosts, even when these are remotely related. Thus mammalian strains are infective to birds. Toxoplasms are, therefore, characterized by a low degree or absence of host-restriction.

Furthermore, strains from different hosts are also immunologically identical, since an animal which has recovered from an infection with one strain is protected against reinfection with others. In view of these facts it is conceivable that the toxoplasms are actually represented by a single species having a wide range of hosts. As this question stands in need of further investigation, the existing species may be retained provisionally as purely descriptive names for strains occurring in different hosts.

The toxoplasms are pathogenic to both naturally and experimentally infected hosts, the infection running an acute or subacute course which usually terminates in the death of the animal. The lesions found in infected animals are small inflammatory foci, with or without necrosis, in the spleen, lung, liver and brain. The parasites are present in the lesions, where they may be found

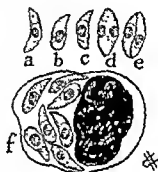


FIG. 38.—*Toxoplasma hominis* ( $\times 2,000$ ). (Original.)

a-e. Free parasites; d, e. Longitudinal binary fission; f. Parasites in macrophage.

enclosed in macrophages, within the pseudocysts, or free in the exudate.

The method of transmission of toxoplasms has not been definitely established. Some observers have suggested that the infection is acquired by ingestion of food contaminated with the infected faeces, others believe that it is transmitted by the droplet method, while others again suspect that some arthropod vector may be involved.

Toxoplasms cannot be cultivated in ordinary artificial media but they can be grown in tissue-cultures (e.g. on developing chick-embryo or in rat heart muscle). They are easily maintained by passages through laboratory rodents, all of which are highly susceptible to infection.

#### xxxvi. *TOXOPLASMA HOMINIS* WOLF *et al.*, 1939

The total number of cases of human toxoplasmosis recorded up to the present, from Europe and North and South America, does not exceed four dozen but there is reason to believe that the incidence of this disease is considerably higher and that it has a wider geographical distribution.

The disease may be acute or chronic. In the former case it appears in three clinical forms, differing in the age-groups affected, in the course of the infection and in the localization of the parasites. In the latter case the infection is usually symptomless.

(1) *Toxoplasmosis in Infants.*—The majority of cases of the disease occurred in new-born infants in which the infection runs an acute and rapidly fatal course, usually lasting not longer than one month. The infantile disease is characterized by involvement of the central nervous system with disseminated inflammatory lesions and symptoms of encephalomyelitis, frequently associated with myocarditis. In the central nervous system the parasites are present in the neuroglia, in the capillary endothelium and in macrophages, while in the myocardium they are found within the cardiac muscle fibres. The parasites are mostly intracellular but occasionally they are free in necrotic patches. The infection in infants is congenital, being acquired *in utero* from mothers with inapparent infections. This is evident from the advanced state of the disease at birth and from the presence of specific antibodies in the mother. The exact method of transmission of the infection to the foetus is not known.

(2) **Toxoplasmosis in Older Children.**—On several occasions the disease was observed in older children who showed symptoms of encephalitis. In these cases it could not be ascertained whether the infection was congenital or acquired after birth.

(3) **Toxoplasmosis in Adults.**—The number of clinical cases recorded in adults is relatively low. The course of these infections was acute and in most cases fatal, the disease resembling typhus fever but being characterized by pneumonitis, with numerous parasites in the pulmonary lesions. While in infants there is extensive destruction of tissues of the central nervous system, in adults the viscera are chiefly involved. There is reason to believe that the infection in adults is acquired after birth.

(4) **Chronic Infections.**—The existence of chronic inapparent infections in persons of different age-groups has been determined by the neutralization test, which is based on the presence of specific antibodies in the blood of human beings suffering from toxoplasmosis. If immune human serum is added to a suspension of toxoplasma-containing material and the mixture is inoculated into the skin of a rabbit, the serum will protect the rabbit from infection. This test was used for the detection of toxoplasmic infection in about 250 persons in the United States, with the result that the presence of neutralizing antibodies was established in about 60 cases. These included mothers of infants who had died of toxoplasmosis, persons showing some of the symptoms associated with the disease, as well as apparently normal individuals. From these tests it is evident that in some cases toxoplasmosis runs a chronic course and does not show any clinical symptoms, the infected persons representing carriers of the disease. It can also be inferred that inapparent infections are much more common and have a wider geographical distribution than the existing records would lead one to suppose.

### Transmission

Practically nothing is known regarding the mode of transmission of human toxoplasmosis. Some observers believe that rodents serve as reservoir hosts and that the infection may be acquired either by eating the incompletely cooked flesh of these animals or through food contaminated with their faeces. In some cases of congenital infantile toxoplasmosis there was actually evidence of close contact between the pregnant mothers and rodents (rabbits and mice).

Since toxoplasms are occasionally found in the blood of the host, it has been suggested that some blood-sucking arthropod might be responsible for the transmission of the disease. In the case of toxoplasmic infection of adults involving the lungs, the parasites have been found within macrophages of the alveoli and bronchioles; it is therefore conceivable that the infection might be conveyed by the droplet method.

### DIAGNOSIS

Up to the present the diagnosis of human toxoplasmosis has been based mainly on *post-mortem* examination of the patient. Though examination of the blood and cerebrospinal fluid occasionally reveals the parasites, their numbers are too scanty for this method to be of any practical value. More satisfactory results are obtained by inoculation of the fluids in question into laboratory rodents, which are susceptible to infection with human strains of the parasite. It has also been suggested that the examination of the sputum might reveal the infection in adult patients.

When found in the muscles, the pseudocysts of *Toxoplasma* bear some resemblance to the cyst-like formations of the parasites in Chagas' disease and in Sarcosporidiosis. However, the leishmanial forms of *Trypanosoma cruzi* can be distinguished from the toxoplasms by the presence of a kinetoplast, while the cyst of *Sarcocystis* can be recognized by the presence of a thick membrane and by internal subdivision into chambers separated by septa (see below).

## CHAPTER 14

### THE SARCOSPORIDIA

THE Sarcosporidia are usually included in the class Sporozoa as a separate order, but the structure and development of these parasites have so many peculiar features that it is best to regard them as a separate group, unattached to any of the existing classes of Protozoa.

Sarcosporidia are represented by a single genus *Sarcocystis*. These parasites occur chiefly in striated and sometimes in unstriated muscles of mammals and, more rarely, in birds, reptiles and fishes. They are especially common in sheep, cattle and horses.

In the muscles the Sarcosporidia appear in the form of elongated cyst-like bodies, known as SARCOCYSTS or "Miescher's tubes" (Fig. 39, a), which measure from a few microns to 5 cm. in length. Externally the sarcocysts are covered by a membrane of several layers, and are divided by partitions (SEPTA) into a number of CHAMBERS. These at first contain a variable number of rounded cells which by repeated binary fission gradually give rise to so-called SPORES. The spores (Fig. 39, b)—sometimes described as "Rainey's corpuscles"—are uninucleate crescentic or bean-shaped bodies, one end of which is rounded and the other pointed. The nucleus, occupying about a quarter of the body, is situated near the rounded end. It has a vesicular structure and contains a karyosome lying near the pole directed to the middle of the body, and a number of scattered chromatin granules. Near the pointed end of the spore there is an oval vacuole, the nature of which is unknown, while the space between this vacuole and the nucleus contains several darkly staining inclusions possibly representing particles of glycogen. The spores, which measure 10–15 $\mu$  in length, are invested in a thin membrane. A fully developed sarcocyst may contain several thousand spores, which are set free when it ruptures and have occasionally been encountered in blood films from mammalian hosts, e.g. cattle.

The further development of these parasites has been studied in mice and rats experimentally infected by feeding them on meat infested with sarcocysts. When swallowed by these animals, the spores lose their membrane and the parasites penetrate into



epithelial cells of the intestinal wall, where they multiply by binary fission. The daughter-individuals find their way into the lymph and blood vessels of the intestinal villi, and are carried by the stream

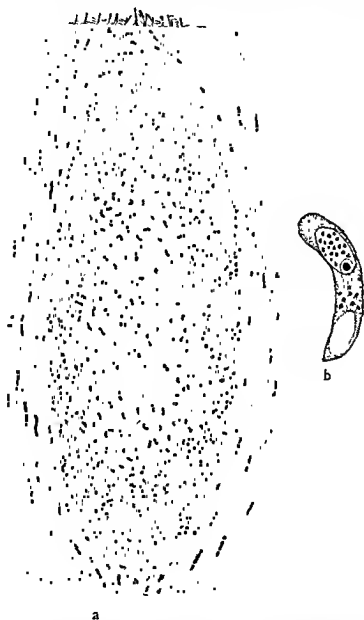


FIG. 39.—SARCOSPORIDIA. (After Wenyon, 1926; and adapted from Alexeieff, 1913; and Reichenow, 1929.)

a. Sarcocyst ("Miescher's tube") in the muscles ( $\times$  ca. 500); b. Single spore ("Rainey's corpuscle") of *Sarcocystis* ( $\times$  1,600).

into the muscle fibres, where they make their appearance about 40 days after infection of the host. In the muscles the parasites settle down, multiply and give rise to the sarcocysts ("Miescher's

tubes"), which gradually grow in size as the parasites increase in numbers.

One of the peculiarities of Sarcosporidia is the ease with which various hosts can be cross-infected. It therefore appears that these parasites are not restricted to any particular host and, since there is little to distinguish the forms occurring in different hosts, it is doubtful if all the species described are valid.

Little is known regarding the pathogenicity of Sarcosporidia but there is reason to believe that in heavy infections they may cause the death of their host. Extracts of Sarcosporidia contain a toxic substance, known as *sarcocystin*, which is probably responsible for most of the pathological effects. When injected into rabbits sarcocystin kills them.

The natural method of transmission of Sarcosporidia is not fully understood. While carnivorous animals may become infected by eating the flesh of infected animals, other animals may acquire the infection through food contaminated with faeces containing the spores.

Infection in man is caused by *Sarcocystis lindemanni* which is morphologically indistinguishable from the species found in lower animals.

#### xxxvii. *SARCOCYSTIS LINDEMANNI* RIVOLTA, 1878

*S. lindemanni* is regarded as pathogenic to man, causing a condition known as sarcosporidiosis, the symptoms of which are not well defined. Sarcosporidial infection has been reported in the muscles of the heart, larynx, tongue and the extremities. The incidence of infection in man is extremely low, the total number of cases reported up to the present not exceeding a dozen. It is thought that human beings acquire the infection from other mammals through ingestion of insufficiently cooked meat or food contaminated with the faeces containing spores. Since the chances of such infection are considerable, it would appear that man is probably an unsuitable host for these parasites.



## PART III

# DIAGNOSTIC METHODS IN PROTOZOAL INFECTIONS

## CHAPTER 15

### INTRODUCTION

FROM the numerous methods of protozoological investigation, a selection has been made of those falling strictly within the scope of this work and representing only the essential methods for the parasitological (or *atiological*) diagnosis of protozoal infections in man. The primary object of this diagnosis is the detection, identification and differentiation of the parasitic protozoa. Since the technique used for the collection and treatment of the material differs considerably, according to whether we are dealing with infections of the blood and the reticulo-endothelial system, on the one hand, or with that of the alimentary tract, on the other, it will be convenient to describe the methods of examination of blood and intestinal protozoa in two independent sections. Separate divisions are devoted to permanent preparations fixed and stained by the "wet" method, to sections of tissues, to cultivation and to examination of insect vectors, with cross-references where these are indicated.

### Equipment

The following list comprises the essential equipment for the microscopic examination of protozoa.

Microscope with  $\times 5$  and  $\times 10$  (= No. 2 and No. 4) oculars ;  $\frac{3}{8}$  in. (low power),  $\frac{1}{2}$  in. (medium power) and  $\frac{1}{4}$  in. oil immersion (high power) objectives ; mechanical stage and substage condenser ; stage and ocular micrometers. The latter can be fixed in a high-power ocular (e.g.  $\times 10$ ) and should be calibrated for each objective and tube-length used. Finally, an electric lamp with a ground-glass screen or a special microscope lamp is required as a source of illumination.



# PART III

## DIAGNOSTIC METHODS IN PROTOZOAL INFECTIONS

### CHAPTER 15

#### INTRODUCTION

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### Other Apparatus

Glass slides and coverslips ( $\frac{7}{8}$  in. square, Nos. 0 and 1); test-tubes, watch glasses, Petri dishes, specimen tubes; glass pipettes and rubber teats; dissecting needles, scissors, scalpels, forceps; platinum loop; wooden spills; spirit lamp or Bunsen burner; syringes; centrifuge.

### Reagents

Ethyl alcohols (70, 90 and 100 per cent.).

Xylol, Canada balsam.

Schaudinn's	} fixatives.
Zenker's	
Bouin's	

Eosin (1 per cent. aq. solution).

Iodine (Weigert's solution).

Normal saline (0.9 per cent. NaCl).

Sodium citrate (6 per cent. saline solution).

Zinc sulphate (33 per cent. solution).

Giemsa's or Leishman's stain.

Field's stain.

Iron alum (4 per cent. aq. solution).

Phosphotungstic acid (2 per cent. aq. solution).

Hæmatoxylin (0.5 or 1 per cent. solution).

Mallory's phosphotungstic hæmatoxylin.

Mayer's acid hæmalum.

Lysol (4 per cent. aq. solution).

### Cleaning Slides

For microscopic work it is important that the slides should be perfectly clean, especially for "dry" and "wet" fixed permanent preparations, for which new unused slides should be employed, while used but cleaned slides can be employed for examination of fresh material.

The simplest method for cleaning new slides is to rub them with one of the household soap pastes or to pass them through alcohol, and then polish with a dry clean rag.

A more thorough method, both for new and used slides, is as follows:—

TABLE 14. DIAGNOSIS OF PROTOZOAL DISEASES

DISEASE	PARASITE	HABITAT	TECHNIQUE
Amebiasis, Amebic dysentery	<i>Entamoeba histolytica</i>	Large intestine, liver, etc.	Faecal examination and culture, scrapings of ulcers (sigmoidoscope), aspiration of liver abscess
Giardiasis	<i>Giardia intestinalis</i>	Small intestine (chiefly duodenum)	Duodenal intubation, faecal examination
Vaginitis	<i>Trichomonas vaginalis</i>	Vagina	Examination of vaginal discharge
Balanitis	<i>Balanidium coli</i>	Large intestine	Faecal examination
Visceral leishmaniasis (Kala-Azar)	<i>Leishmania donovani</i>	Reticulo-endothelial system	Examination and culture of blood and of material from puncture of inguinal glands, sternum, spleen and liver
Cutaneous leishmaniasis (Oriental Sore)	<i>Leishmania tropica</i>	Sores and ulcers of skin	Examination and culture of material from puncture of sores and mucous membranes
Mucocutaneous leishmaniasis (Espundia, etc.)	<i>Leishmania brasiliensis</i>	Ulcers of skin, oral and nasopharyngeal mucosa	Examination and culture of material from puncture of sores and mucous membranes
Sleeping sickness	<i>Trypanosoma gambiense</i> , <i>T. rhodesiense</i>	Blood, lymph glands, nervous system	Examination of blood, juice of cervical lymph glands (puncture), lumbar puncture, inoculation of susceptible mammals
Chagas' disease	<i>Trypanosoma cruzi</i>	Blood, muscles, etc.	Examination and culture of blood, biopsy of muscle, inoculation of susceptible mammals, xenodiagnosis
Malaria	<i>Plasmodium vivax</i> , <i>P. falciparum</i> , <i>P. malarie</i> , <i>P. ovale</i>	Blood, spleen, liver, brain, etc.	Examination of blood (thick and thin films), sternal puncture
Toxoplasmosis	<i>Toxoplasma hominis</i>	Chiefly central nervous system	Inoculation of laboratory animals with cerebrospinal fluid or blood



**Cleaning Fluid :** potassium dichromate 100 g. ; sulphuric acid (pure) 100 ml. ; distilled water 1 litre.

**Procedure :** (1) Half fill large evaporating basin with cleansing fluid, introduce slides one at a time to ensure contact with fluid ; (2) Soak slides for 24 hours or boil for 10 minutes, keeping slides completely immersed ; (3) Wash in running water until cleaning fluid is completely removed (yellow colour disappears) ; (4) Wash in 3 changes of distilled water ; (5) Drain off water, add methylated spirit, and rinse slides ; (6) Dry slides with clean, grease-free duster ; (7) Before use, flame slides to remove moisture.

**NOTE :** Do not handle slides but use grease-free forceps.

### Disinfection

When handling infective material, especially faeces, the worker is constantly exposed to the risk of infection by contamination of the hands. It is, therefore, necessary to observe all the usual bacteriological precautions in the course of the work. For this purpose a jar containing 4-5 per cent. solution of lysol should be available and into this all slides, specimen tubes and other objects (e.g. matches, wooden spills, etc.), which have been used in the course of examination and preparation of the material, should be put. The hands can also be disinfected by immersion in a solution of the same strength.

### Material

Table 14, containing information regarding the habitat of pathogenic human protozoa, source of material and the technique used for their detection, is intended to serve as a guide to the parasitological diagnosis of protozoal diseases.

## CHAPTER 16

### TREATMENT OF MATERIAL FOR INTESTINAL PROTOZOA

IN all intestinal infections with protozoa the causal organisms are sooner or later voided in the stools. They can therefore usually be detected by microscopic examination of the faeces, but in metastatic infections in amœbiasis (e.g. liver abscess), it may be necessary to resort to operative procedures. The technique of faecal examination is the same for all intestinal protozoa.

#### COLLECTION

First comes the collection of material. In this connexion it is important that the following rules should be observed.

(1) The stools should be as fresh as possible when examined, since the active forms of the protozoa die rapidly outside the body.

(2) Stools should be collected in clean, dry receptacles. The presence of antiseptics and of urine kills the protozoa (especially the active forms), while if tap-water is used to dilute the faeces or is left in the bed-pan after flushing, it may contain extraneous, free-living coprozoic protozoa, the presence of which in the faeces may occasionally lead to wrong diagnosis. (There are many instances of coprozoic protozoa having been described as new human parasites.)

(3) The stool should preferably be natural, i.e. not obtained with the help of purgatives, as some of these, especially oily substances, may affect both the parasites and the microscopic picture; but if the consistency of the stool is formed, a saline purgative (magnesium or sodium sulphate) may be used to reveal the active protozoa (e.g. amœbæ).

(4) If possible, the whole stool should be seen as it is collected in the bed-pan; and, if it is not homogeneous, samples should be taken for examination from different portions of the stool (e.g. formed faeces, patches of mucus, fluid, etc.).

(5) If a faecal specimen is not obtainable in the normal way, a sample can be collected by inserting into the rectum a rubber catheter and rotating it: faecal matter adhering to the "eye" of

the catheter may provide sufficient material for microscopic examination.

(6) For microscopic examination a faecal specimen is placed in a suitable receptacle, such as (a) a specimen tube (3 × 1 in.) with a spoon attached to the cork, (b) a waxed cardboard or sputum container with a screw-on lid, etc. The amount of faeces should not be excessive. Material from the container should be examined immediately; if this is impossible, it can be sent to a laboratory. If active protozoa are present, the interval before examination should not exceed 1–2 hours and the container should be kept cool, but if the faeces contain cysts, the container can be sent by post. It should always bear a label with the names of patient and sender, date of collection, place, and suspected condition.

#### Special Methods in Amœbiasis

**Use of Purgatives.**—If the examination of a stool does not reveal *Entamœba histolytica*, the amœbæ can sometimes be demonstrated after administration of a saline purgative, such as magnesium or sodium sulphate. The solid portion of the discharge is discarded and particles of mucus are removed from the fluid with a pipette and examined microscopically.

**Sigmoidoscopy.**—The sigmoidoscope is employed in the clinical diagnosis of amœbiasis for demonstrating the presence of the characteristic ulcers in the rectum and sigmoid colon. If lesions are present, material can be removed from them with the aid of a swab and examined microscopically for the presence of *Entamœba histolytica*. However, this method should not be used for routine purposes in the protozoological diagnosis but only if repeated examinations of the faeces fail to reveal the parasite.

**Liver Abscess.**—In amœbic abscess of the liver, *E. histolytica* occurs chiefly in the tissues of the abscess wall, whereas in its contents amœbæ are very scanty, especially if there is a secondary bacterial infection. The amœbæ should be sought for in the pus collected in the course of the exploratory puncture of the liver, carried out for the clinical diagnosis of the condition, and subsequently after aspiration and drainage of the abscess. In the latter case one can only expect to discover the amœbæ in the last portion of pus to be withdrawn. The procedures themselves are outside the scope of this book.

## EXAMINATION

For microscopic examination the following reagents are required :

(1) 1 or 2 per cent. aqueous solution of eosin ; (2) normal saline : 0.9 per cent. NaCl ; (3) iodine solution (2 I + 4 KI + 100 H<sub>2</sub>O : Weigert's or Lugol's).

A faecal preparation—in any of these solutions—is made as follows : a drop of the fluid is placed on a slide, and with a wooden match or spill a small grain of faecal matter is teased up in the fluid, to produce a suspension, after which a coverslip is applied to the specimen (and pressed down, if necessary).

Care should be taken that the mounted preparation is neither too thick nor too thin, and that the drop of fluid is not too abundant. The right proportions are arrived at by practice. It should be possible to read small print through a good faecal preparation.

The different solutions are used for the following purposes :—

(1) Eosin has the property of staining everything in the stool, except living protozoa, pink ; therefore, the eosin preparation will reveal the presence of protozoa (and their cysts) as colourless, pearl-like bodies against a pink background. Eosin does not kill the protozoa, therefore in fresh stools the active forms may be motile. On the other hand, if the protozoa are already dead they will stain pink. On this account, the eosin solution also serves as a test of viability (e.g. for testing the effect of drugs or disinfectants). As a rule, the eosin preparation does not reveal much structure in the protozoa and some of these may be difficult to identify.

The main object of the eosin preparation is merely to show whether or not protozoa are present. It also gives an idea of the degree of infection, and is particularly useful for the detection of cysts of amœbæ (Pl. I).

(2) Saline is used for the same purposes as the eosin solution, but as both the background and the organisms are colourless, the protozoa are more difficult to detect. Saline preparations are suitable for observation of the active, motile stages of protozoa.

The structure of active flagellates can also be studied by killing them with *osmic vapour*. The slide with a drop of saline containing the flagellates is inverted over a bottle, in which there is a small amount of 2 per cent. osmic acid, and exposed for about 10 seconds, after which the preparation is examined under a coverslip.

(3) Iodine kills any protozoa that may be present, and stains them and everything else more or less uniformly yellow-brown. At the same time iodine reveals in the protozoa various structures which are not seen in the eosin or saline preparations, but which are of diagnostic value, e.g. the nuclei in cysts of amœbæ, brown-staining glycogen in these cysts, etc. (Pl. I). The iodine preparation thus permits the identification and differentiation of protozoa, the presence of which has been detected in the eosin or saline preparation.

It is advisable in all cases of faecal examination to employ a set of two solutions: eosin (or saline) and iodine. Faecal preparations in eosin and saline are first examined under low power ( $\frac{3}{8}$  in. objective) of the microscope; if bodies suspected to be protozoa are detected, they are examined under higher powers ( $\frac{1}{8}$  in. objective and  $\frac{1}{2}$  in. objective with oil immersion), which are also employed for the examination of the iodine preparation (see Chapter 4).

If cysts are present, but too scanty for identification in the ordinary faecal smear preparation, they can be concentrated by one of the following zinc sulphate flotation methods.

## FAUST'S ZINC SULPHATE FLOTATION METHOD

### (1) WATSON'S MODIFICATION

(a) A 33 per cent. aqueous solution of zinc sulphate is made ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ : crystalline; specific gravity about 1.18).

(b) A sample of the stool to be investigated, of about the size of a pea, is placed in a glass centrifuge tube with distilled water and broken up with the aid of a thin wooden rod, to form a fine suspension.

(c) The suspension is centrifuged for 3 minutes at 1,500 revolutions per minute, using an ordinary laboratory centrifuge with a radius of  $5\frac{1}{2}$  in. Supernatant fluid is then removed and the process is repeated, usually twice, until the supernatant fluid at the end of the last spin is clear.

(d) After removal of the clear supernatant fluid, some zinc sulphate solution is poured into the tube and the packed sediment is broken up into a uniform suspension. More solution is then added until the edge of the meniscus is level with the top of the glass tube.

(e) A chemically clean circular coverslip, of slightly greater diameter than the glass tube, is then lightly smeared with a thin film of Mayer's albumen (a mixture of equal parts of egg-white and glycerine) and pressed firmly and symmetrically on to the top of the glass centrifuge tube, care being taken that no air-bubbles are trapped beneath it.

(f) The suspension is again centrifuged for 3 minutes at 1,500 revolutions per minute.

(g) When the centrifuge stops, the superimposed coverslip is carefully and rapidly lifted off the top of the tube and placed, prepared surface downwards, on a drop of Weigert's iodine on a slide, when it is ready for microscopic examination.

## (2) OTTO, HEWITT AND STRAHAN'S MODIFICATION

(a) A small portion of faeces is placed in a cylindrical glass tube (about  $2 \times \frac{3}{4}$  in.) and emulsified with a small amount of zinc sulphate solution (prepared as in Watson's modification: 1, a).

(b) The tube is filled to the top with more solution; larger particles of faeces are removed from the surface with the help of a platinum loop, then more solution is added until the edge of the meniscus is level with the top of the tube.

(c) A coverslip ( $\frac{1}{8}$  in. square) is placed over the top of the tube and left for 20-30 minutes: the cysts will rise and become attached to the undersurface of the coverslip.

(d) A drop of Weigert's iodine solution is placed in the middle of a slide.

(e) With the help of forceps the coverslip is lifted from the tube and applied (film downwards) to the drop of iodine on the slide.

(f) The preparation is then examined microscopically.

The second concentration method is much simpler than the first and can be used if a centrifuge is not available. Though the results obtained by the simplified method are quite satisfactory, the highest concentration of cysts is obtained by the first method.

## Fallacies in Faecal Examination

Human faeces contain numerous structures which the inexperienced worker might mistake for protozoa, especially their cysts. Among these the following should be mentioned:—

(1) Tissue cells, e.g. epithelial cells and leucocytes.

(2) Microorganisms, the most common and important one being a unicellular fungus, *Blastocystis hominis* (Fig. 12, 1, m) which has a rounded body (8-14 $\mu$ ), consisting of an annular layer of cytoplasm with one or more nuclei and a large central sphere or vacuole, not giving the glycogen reaction (brown colour) with iodine.

(3) Undigested particles of food.

(4) Air-bubbles and drops of oil: these can be distinguished from the protozoal cysts by altering the focus of the microscope. Cysts are whitish and do not exhibit a bright point of light when out of focus, whereas air-bubbles and oil-drops show this phenomenon.

(5) In stained preparations, polymorphonuclear leucocytes are sometimes mistaken for quadrinucleate cysts of *Entamoeba histolytica*, especially when the nucleus of the leucocyte consists of four lobes.

#### Permanent Faecal Preparations

Permanent preparations of intestinal protozoa are made as follows. If the faeces are solid or too thick, a sample is diluted with normal saline until it has a consistency of thick pea-soup; if the faeces are semi-solid or liquid, they need not be diluted. With a match or a platinum loop a small portion of the faeces is picked up and smeared rapidly in a thin film on a coverslip, which is held between thumb and index finger of the left hand. Without allowing the smear to dry, the coverslip is immediately dropped film downwards on to the surface of the fixing fluid.

For the choice of fixative and further procedure in the fixation, staining and subsequent treatment of the preparations, the reader should consult Chapter 18.

## CHAPTER 17

### TREATMENT OF MATERIAL FOR BLOOD PROTOZOA

THE technique for examining blood protozoa (malaria parasites, leishmanias and trypanosomes) applies both to the stages found in the blood itself and to those which occur in other tissues. A graphic representation of the sites, from which the material for the parasitological diagnosis of protozoal diseases of the blood and the reticulo-endothelial system is obtained, is given in Fig. 40.

#### Examination of Fresh Blood

A drop of blood is taken either from the lobe of the ear or from the distal phalanx of a finger (Fig. 40, a, b). The site should first be wiped with alcohol or ether and allowed to dry, after which a puncture is made with a flat needle or scalpel.

A small drop of blood is then placed on a slide, a coverslip is applied to it and slightly pressed to ensure even distribution of the blood film. The fresh preparation is examined under low ( $\frac{3}{4}$  in. objective) and medium ( $\frac{1}{4}$  in. objective) powers of the microscope. Fresh blood is usually examined for the detection of trypanosomes.

#### Centrifugation of Blood

In the case of Sleeping Sickness, when the number of trypanosomes is too scanty to be seen in a fresh blood film, the trypanosomes can be concentrated by centrifuging the blood as follows: 9 ml. of blood are taken from a vein (median basilic: Fig. 40, c) into a 10 ml. syringe containing 1 ml. of 6 per cent. saline solution of sodium citrate. The citrated blood is then expelled into a centrifuge tube and centrifuged thrice. First for about 7 minutes, starting at 900-1,000 revolutions per minute and gradually increasing the speed to 1,500. The plasma containing white cells and trypanosomes is then decanted into a second tube and centrifuged at 1,500 revolutions for 10 minutes, thereby throwing down the white cells and platelets. When numerous, the trypanosomes can be found in the sediment of the second tube. The serum after the second centrifugation is decanted into a new tube and centrifuged for the third



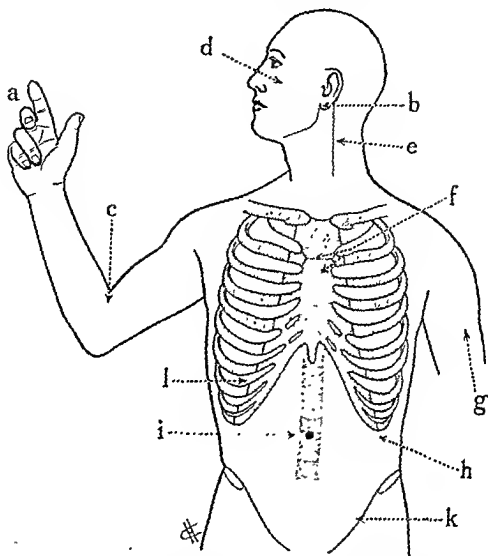


FIG. 40.—COLLECTION OF MATERIAL FOR PROTOZOOLOGICAL EXAMINATION IN MALARIA, TRYPANOSOMIASIS, LEISHMANIASIS AND TOXOPLASMOSIS. (*Original*.)

a, b, Blood from finger and ear-lobe; c, Blood from vein; d, Puncture of Oriental Sore; e, Puncture of cervical gland; f, Sternal puncture; g, Puncture of primary skin lesion in Sleeping Sickness; h, Spleen puncture; i, Lumbar puncture; k, Puncture of inguinal gland; l, Liver puncture.

Application: 1. Malaria: a, b, f; 2. Sleeping sickness: a, b, c, e, g, i; 3. Chagas' disease: a, b, c; 4. Kala-azar: a, b, c, f, h, k, l; 5. Oriental Sore: d; 6. Toxoplasmosis: i.

time at 1,800–2,000 revolutions for 15–20 minutes. The deposit from the third centrifugation, containing rare leucocytes and red cells, platelets and trypanosomes, is examined immediately between slide and coverslip under medium power ( $\frac{1}{2}$  in. objective).

### Preparation of Blood Films

The preparation of blood films is shown in Fig. 41. There are two kinds of blood films: thin and thick.

(1) For a thin film a drop of blood, not larger than the head of a pin, is taken on the edge of one slide (the spreader), which is then placed with the drop downwards—at an angle of about  $45^{\circ}$ —against another slide, lying horizontally, at a distance of about  $\frac{1}{2}$  in. from its right end. The blood is allowed to run along the edge between the two slides, the spreader is lowered to an angle of about  $30^{\circ}$  and pushed gently but firmly to the left end of the other slide until a film is made. The blood is thus not pushed in front of the spreader but dragged behind it. As soon as the film is made, it is dried rapidly in the air (without flaming) by waving the slide backwards and forwards.

A perfect thin blood film should consist of a single layer of red blood corpuscles and its margins should not reach the sides of the slide.

(2) For a thick film, a drop of blood (somewhat larger than that for a thin film) is collected from the puncture on to a slide and spread (with a needle or the corner of another slide) in a flat spiral, without gaps, over an area covering three times its original diameter. The film is left to dry in a horizontal position, and should be protected from flies and dust by covering the slide with a Petri dish. Drying may be accelerated by putting the slide in an incubator. When the film is quite dry, the slide can be passed *rapidly* two or three times through a spirit or Bunsen flame.

A good thick film should be not more than 10–20 times the thickness of a thin film, and it should be possible to read newsprint or to see the hands of a wrist-watch through the dry preparation.

(3) Thick and thin films can be combined on the same slide. In this case one drop of blood is placed  $\frac{1}{2}$  in. and another 1 in. from the end of the slide. The latter drop is made into a thin film, while the former is made into a thick film. Slides with combined films are economical and obviate the risk of confusing the cases to which they belong. For staining methods see Chapter 18.

Application.—A thick blood film represents a concentration method and is used primarily for detecting the presence of parasites. Since a thick film contains in a given area so much more blood

microscopically for leishmanias under a  $\frac{1}{2}$  in. objective with oil immersion.

### Puncture of Oriental Sore

In non-ulcerating cutaneous leishmaniasis (Oriental Sore) the parasites can be detected in the fluid obtained by puncture of the sore, but in ulcerating forms the superficial part of the sore contains many pus cells and extraneous microorganisms with very scanty leishmanias. In such cases the parasites are usually found in the deeper parts of the ulcer (Fig. 40, d). To discover the parasites the indurated red margin of the skin surrounding the ulcer is first sterilized with tincture of iodine, then, with a needle, a puncture is made through this area in the direction of the base of the ulcer, after which the needle is withdrawn and in its place a sterile capillary pipette is introduced by boring into the puncture. Part of the material from the sore, which enters the pipette by capillary attraction or can be aspirated, is blown on to a slide and made into a smear which is then dried and stained with one of the Romanovsky stains (Chapter 18). Another portion of the material can be inoculated into suitable media for cultivation (Chapter 19.)

### Puncture of Primary Nodule in Trypanosomiasis

In Sleeping Sickness trypanosomes can be detected in the primary skin lesion (Fig. 40, g) by pricking the indurated edge of the nodule with a needle and examining the fluid exudate microscopically in a fresh preparation and/or in a smear stained by one of the Romanovsky methods.

### Spleen Puncture

This method is employed for the detection of leishmanias in Kala-Azar. The abdomen of the patient is fixed with a binder, and 1 hour before the operation an injection of 0.01 grain (0.00065 g.) of atropine is given and the site of the puncture is infiltrated with novocain. The lower border of the spleen is kept steady by the hand, pressing it upwards to the diaphragm. The hypodermic needle (Maw's size 10 with a shaft 4 cm. long or Napier's spleen-puncture needle) and barrel of syringe (5 ml.) should be dry. When the spleen is enlarged puncture is made  $\frac{1}{2}$  in. below the costal margin

(Fig. 40, h), but when it is small, the 8th or 9th intercostal space may be chosen. The site is cleaned with alcohol and painted with tincture of iodine. With the piston of the syringe drawn half-out, the needle is plunged to a depth of 3, 6 or 8 cm. Aspiration of material from the spleen is continuous, accompanied by gradual withdrawal of the needle until it is near the abdominal wall, when suction is stopped, and the syringe is removed. Part of the contents of the needle is blown out on to a slide, smeared, dried, stained by one of the Romanovsky methods, and examined under  $\frac{1}{2}$  in. objective with oil immersion. Another portion may be inoculated into a tube with culture medium (Chapter 19).

### Liver Puncture

As a diagnostic method for detecting leishmanias in Kala-Azar, liver puncture is of little practical value and is now almost entirely superseded by sternal puncture. The procedure is the same as in spleen puncture, the site being the 7th or 8th intercostal space in the mammary line (Fig. 40, l).

### Lumbar Puncture

This method is used for the detection of trypanosomes in the later stages of Sleeping Sickness. The patient is either lying on his side on a couch, with knees and chin approximated, or he is seated on a stool, bending forward as far as possible. The puncture is made through the interspace between the laminae of the 3rd and 4th lumbar vertebrae, which is located by drawing, with a swab dipped in alcoholic iodine, a horizontal line across the back at the level of the highest points of the iliac crests (Fig. 40, i). Local anaesthesia may be produced at the site of puncture by the injection of 4 per cent. sterile novocain. A special needle (usually of platinum-iridium or nickel) is employed, 8-10 cm. long, with a bore of 1-1.5 mm., having a bevelled end and provided with a stylet. The needle is inserted firmly in the midline and pushed forward, until it is felt, by a sudden cessation of resistance, to enter the spinal canal. The stylet is then removed, and the fluid, if under normal pressure (100-200 mm.), should drip from the needle.

After discarding the first few drops, which are usually blood-stained, 5 ml. of the cerebrospinal fluid are collected into a centrifuge tube and spun at 1,800 revolutions per minute for 20 minutes.

After most of the supernatant fluid has been decanted, the sediment with some of the remaining fluid is shaken and removed to a slide in two drops which are allowed to dry. The trypanosomes will accumulate along the outer edge of the drop, while the cells are distributed all over the area. The dried preparation is fixed in absolute alcohol for 5 minutes and stained with Giemsa's stain in the usual way. The edge of the stained film should be searched for trypanosomes under a  $\frac{1}{2}$  in. objective with oil immersion. A count of the trypanosomes in the two drops gives the total number present in the original volume of cerebrospinal fluid.

For cell counts the sediment is examined in the counting chamber of a haemocytometer.

### Sternal Puncture

This method is employed mainly for the detection of leishmanias in Kala-Azar and sometimes also for malaria parasites.

One hour before the operation the patient receives  $\frac{1}{4}$  grain (0.016 g.) of morphia. The skin, subcutaneous tissues and the periosteum of this area are infiltrated with novocain.

The operation is carried out with a needle (18 gauge spinal puncture needle 3-4 cm. long, or Witt's or Salah's sternal puncture needle) provided with a moveable guard and with an obturator stylet inserted into it. With the patient lying on his back, the puncture can be made in two different ways (Fig. 40, f): (1) the needle is plunged into the cartilage at the sternomanubrial junction, then lowered to an angle of about  $30^{\circ}$  and pushed upwards, piercing the manubrium from below to a depth not exceeding 1.5 cm., or (2) it is introduced into the body of the sternum slightly to one side of the middle line at the level of the second intercostal space.

The stylet is then pulled out, a syringe is attached to the needle, and some bone-marrow is drawn into the barrel. The syringe is removed from the needle, and the aspirated material is transferred to a slide, smeared, and stained by one of the Romanovsky methods. In the meantime the stylet is replaced in the needle, which is withdrawn, and the puncture wound is sealed with collodion.

## CHAPTER 18

### PERMANENT PREPARATIONS

FOR permanent preparations of parasitic protozoa it is necessary, first to fix the parasites, then to stain them. The best results, from the cytological point of view, are obtained by the so-called "wet" method, in which the preparation is carried through the successive stages of the operation, from fixation to the final mounting, without being allowed to dry in the process. However, in the case of blood protozoa and some other parasites, satisfactory results can be produced by the so-called "dry" method, in which smears or films of the material containing the parasites are dried before and after staining, and need not be mounted. The "dry" method is used almost exclusively for the examination of malaria parasites, trypanosomes and leishmanias.

#### PREPARATIONS MADE BY THE "DRY" METHOD

The treatment of blood films and tissue smears employed in the diagnosis of protozoal infections of the blood and other tissues is known as the "dry" method, since it is used for preparations which have first been fixed by drying in the air before staining (see below). Such preparations are stained by one of the following modifications of Romanovsky's technique: Leishman's, Wright's, Giemsa's or Field's stains.

These stains are used for both thick and thin blood films, as well as for smears, the method of staining in the two cases differing slightly, owing to the dehaemoglobinization of the red blood corpuscles in thick films, as the result of which these cells remain unstained. This treatment renders the film sufficiently transparent to reveal only the stained parasites and leucocytes lying at different levels. Unless laked, the erythrocytes would stain deeply and obscure the parasites.

#### Distilled Water

When staining by any of these methods (except Field's stain) it is essential that the distilled water used for diluting the stain should be neutral or slightly alkaline (pH 7.0-7.2). Water fresh from the distiller is always neutral but after standing for some time in the

laboratory it invariably becomes acid in reaction. When acid water is used for staining it produces defective preparations. However, it is quite easy to ensure that the water is neutral. The methods employed are as follows :—

(1) Immediately after distillation, small bottles with glass stoppers are filled *to the top* with the water, which will remain neutral for quite a long time. The contents of each bottle should be used once only.

(2) If distilled water has been stored with access of air (and carbon dioxide) it can be neutralized by the following methods :—

(a) By boiling the required amount of water for 10 or 15 minutes in an open beaker to expel  $\text{CO}_2$ , and then allowing to cool. The beaker should be of hard glass (e.g. Pyrex) ; if boiled in soft glass-ware, the water may be too alkaline. In a flask it may be necessary to boil for more than 30 minutes before the water is neutral.

(b) By adding to the water 1 or 2 drops of saturated aqueous solution of lithium carbonate, stirring and testing the resulting reaction by addition of a few drops of 0.02 per cent. of phenol red to about 5 ml. of the water. The process is repeated until the water is neutral or slightly alkaline (pH 7-7.2). If, on addition of lithium carbonate, the water becomes too alkaline, the pH can be lowered by addition of some of the acid water from stock. The reaction of the water can be determined by comparison with a colour chart or a standard set of tubes for phenol red.

In an emergency, if distilled water is unobtainable, clean, filtered *rain water* may be used for diluting the stain.

### LEISHMAN'S STAIN

#### Preparation

The stain is available in the form of tablets or powder. First 10 ml. of pure methyl alcohol is poured into a bottle, and 0.015 g. of the powdered stain is added to the alcohol. The bottle is then stoppered and shaken vigorously for a few minutes, the shaking being repeated several times in the course of the same day and on the following day.

The stain is ready for use 24 hours after preparation, and will keep for several months in cool weather or stored in an ice chest, but in warm weather it is liable to deteriorate after some weeks.

## Method of Staining

## (a) THIN FILMS

(1) The slide is placed on a staining rack, film-side up ; (2) the film is covered with a measured number of drops (5-10) of undiluted Leishman's stain and left for 30 seconds (the methyl alcohol in the stain fixes the film) ; (3) double the quantity of distilled water (10-20 drops) at pH 7-7.2 is added to the slide and is mixed with the stain by drawing and expelling the fluid through a pipette ; (4) staining is continued for 15 to 20 minutes, the time varying with different batches of stain ; (5) the slide is then brought under an open tap in a *horizontal* position, tilted and flushed for an instant in a gentle flow of water ; (6) finally the slide is placed in an upright position to drain and dry.

## (b) THICK FILMS

(1) The slide with the dry, unfixed film is placed upright in a glass cylinder containing distilled water and left until all the hæmoglobin is removed (about 10 minutes) and the film is white ; (2) the slide is then placed in an upright position to dry, after which it is treated as in the case of thin films (see a, 1-6).

## WRIGHT'S STAIN

This stain, which is used chiefly in America, is practically identical with Leishman's stain. It is prepared in the same way by dissolving 0.05 g. of the powdered stain in 10 ml. of pure methyl alcohol.

Thin blood films are stained by putting the undiluted stain on the film, leaving it for 1 minute, then adding distilled water, 1 drop at a time, until a metallic scum appears. Staining is continued for 5-20 minutes, according to the intensity desired, after which the slide is rinsed in running distilled water, and left to drain and dry.

## GIEMSA'S STAIN

This stain is obtainable in ready-made solution and keeps indefinitely.

## Method of Staining

## (a) THIN FILMS

(1) The slide is placed upright in a jar with absolute ethyl or methyl alcohol and fixed for 3 minutes, after which it is allowed to



dry ; (2) the stain is diluted by adding 1 drop of the stock solution to each 1 ml. of distilled water (at pH 7-7.2), a measured volume of which is prepared (about 5 ml. per film is required) ; (3) the diluted stain is either poured into a shallow dish and the slide placed in it film downwards, or the slide is placed on a staining rack, film-side up, and covered with the stain ; (4) the slide is stained for 20-40 minutes, according to the desired intensity ; (5) then the slide is flushed for an instant in a gentle flow of tap-water, and (6) placed in an upright position to drain and dry.

### (b) THICK FILMS

These are left *unfixed* and placed directly into the dilute stain, prepared as for thin films (see *a*, 2-4), in which dehaemoglobinization and staining proceed simultaneously. When stained, the slide should be immersed for an instant in a dish of tap-water, then placed upright to drain and dry.

### FIELD'S STAIN

This is a rapid method for staining thick blood films (without fixation).

#### Preparation

The following three solutions are made up :—

(a) Methylene blue (medicinal) 0.4 g.

Azure I 0.25 g.

Buffered water (see *b*) 250 ml.

(b) Buffered water :—

Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$  : anhydrous)  
10.0 g.

Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$  : anhydrous)  
12.5 g.

Distilled water 1 litre.

(c) Eosin 0.5 g.

Buffered water (see *b*) 250 ml.

The stains (*a* and *c*), when kept in stoppered glass cylinders, can be used for several weeks, but buffered water (*b*) should be renewed more frequently.

### Method of Staining

- (1) Dip slide in *a* for 1-3 seconds,
- (2) Rinse in *b* for 2-3 seconds,
- (3) Dip in *c* for 1-3 seconds,
- (4) Rinse gently in tap-water for 2-3 seconds,
- (5) Place slide upright to drain and dry.

### General Remarks on Staining

When flushing the stained slides with tap-water care should be taken not to prolong the washing, otherwise the parasites may become decolorized.

Thick films should be treated very gently at all stages, otherwise they may become detached from the slide.

Heat must not be used to dry stained films, but the process can be accelerated by wiping off excess of water from parts of the slide not occupied by the film and waving it in the air.

As regards the choice of stain to be used, Field's technique for thick films is a rapid method, enabling a diagnosis to be arrived at in a few minutes; and while equally good results can be obtained with either Leishman's or Giemsa's stain, the latter is more suitable for work in the tropics than the former, which is liable to deteriorate.

### Fallacies in Dry Films

The method of preparation of dry blood films and tissue smears, involving the use of distilled water and exposure of uncovered slides to the air, renders them liable to contamination with extraneous organisms and other objects, which might be—and actually have been—mistaken for organisms present in the material examined.

Some of the sources of error may be purely mechanical, due to scratches on old and dirty slides in which the stain becomes deposited. Others may be due to contamination of the film with extraneous microorganisms, pollen grains, etc., originating from the air or deposited by flies settling on the film. Distilled water used for diluting the Romanovsky stains is another common source of error. If stored for some time, especially in the tropics, it is liable to be contaminated with bacteria and free-living protozoa, which will stain and adhere to the film. When the film is examined the presence of these structures may give rise to confusion and lead to errors of diagnosis, unless their true nature is recognized.

## PREPARATIONS MADE BY THE "WET" METHOD

The "wet" method of making permanent preparations is the one generally employed in zoological and histological work and is the only one which reveals the exact structure and cytological details of animal cells and tissues. Of the great variety of fixatives and stains used in protozoological investigation only those will be dealt with which do not require an elaborate technique and which produce well-stained preparations with the least difficulty. They include both methods for rapid diagnosis and those which can be used when there is no urgency. Most of the methods described can be used both for smear preparations and for sections.

Smear preparations are of special importance in demonstrating the structure of intestinal protozoa (e.g. in faecal smears) and are employed both for general morphological and for cytological purposes. They are particularly useful for differentiation of the species of amœbæ, when there is any doubt about their identity after examination of fresh faecal preparations.

Sections are of particular value for determining the localization of the parasites in human tissues as well as for the study of the lesions produced by them, e.g. in leishmaniasis, trypanosomiasis, amœbiasis and balantidiosis. Sections of insect-vectors—sandflies, mosquitos, tsetse-flies and bugs—reveal the developmental stages of the blood parasites and their distribution in the intermediate host.

The treatment of the material involves the following successive stages: (a) fixation, i.e. rapid killing of the protozoa and preservation of their normal appearance and structure, (b) elimination of the fixing fluid from the preparation, (c) staining, (d) dehydration, (e) clearing and, finally, (f) mounting.

### 1. Fixation

Smears.—The best fixative for ordinary smear preparations is Schaudinn's fluid, which is made up as follows:—

### SCHAUDINN'S FLUID

#### Composition

Corrosive sublimate ( $\text{HgCl}_2$ ), saturated aqueous solution, 100 ml.; absolute ethyl alcohol 50 ml.; glacial acetic acid 7.5 ml.  
This fixative will keep indefinitely.

### Procedure

In the case of ordinary smear preparations the procedure is as follows: the fixing fluid is placed in a Petri dish or watch-glass, according to the number of preparations made at the same time; then, on a coverslip held in the left hand between thumb and index finger, a thin smear of material containing the protozoa—if necessary, diluted with normal saline—is made with the aid of a match or a platinum loop, and before the film can become dry, the coverslip is immediately dropped *film downwards* upon the surface of the fixing fluid, on which it will float for some time. It is later turned over (with a dissecting needle or spatula) and all subsequent operations are carried out with the coverslip lying *film upwards*.

The film is left in the fixative, which is covered to prevent evaporation, up to 30 minutes, after which the coverslip is transferred to a dish containing 70 per cent. alcohol, to which a little iodine solution (Weigert-Lugol's)—sufficient to produce the colour of sherry—has been added: in this fluid the coverslip can be left for 20–60 minutes, in the course of which the sublimate is removed from the film. The coverslip is then transferred to pure 70 per cent. alcohol (2 changes): in this the preparation can be kept before staining for 1 hour or for an indefinite period. If the coverslip preparations are to be stored, they can be stacked in stoppered glass cylinders, containing 70 per cent. alcohol, and covered with cotton wool, to prevent breakage.

Smear preparations can also be fixed in Bouin's fluid, as described below.

Sections.—For the preparation of material for sections, a portion of the tissue infected with protozoa is cut out and dropped into a specimen tube or other suitable glass receptacle containing the fixative. The best all-round fixatives are Zenker's fluid and Bouin's picro-formol (aqueous or alcoholic), which are made up and employed as follows.

### ZENKER'S FLUID

#### Composition

1.5 g. corrosive sublimate ( $\text{HgCl}_2$ ) and 5 ml. glacial acetic acid are dissolved in the following (Müller's) solution:—

Potassium bichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ )	.	.	2.5 g.
Sodium sulphate ( $\text{Na}_2\text{SO}_4$ )	.	.	1.0 g.
Distilled water	.	.	100 ml.

### Procedure

In this reagent the tissues are fixed for from 3 to 12 hours, depending upon the size of the tissue, after which they are washed in running tap-water overnight. This can be done by placing the fixed material in a glass cylinder, e.g. a lamp glass, one end of which is closed with muslin: the water enters through the open end and runs out through the closed end into a bowl, from which it overflows. The tissue is then removed to 70 per cent. alcohol to which Weigert's iodine has been added, as in the case of Schaudinn's fluid (see above), and finally it is transferred to pure 70 per cent. alcohol, in which it is left overnight or longer.

The subsequent treatment of tissues fixed for sections (embedding in paraffin wax, cutting sections, etc.) is outside the scope of this work and will be found in handbooks of histology.

### BOUIN'S Picro-FORMOL

Bouin's picro-formol is an excellent penetrating fixative both for smear-preparations and for tissues prepared for section. It can be employed in two forms: (a) the original aqueous solution and (b) Dobell's alcoholic solution.

#### Composition

For (a) Picric acid, saturated aqueous solution	} 75 ml.
For (b) Picric acid, saturated solution in 90 per cent. alcohol	
The remaining constituents are the same for both variants:	

Formol (40 per cent. formaldehyde)	. 25 ml.
Acetic acid (glacial)	. . . . 5 ml.

### Procedure

Smear-preparations are fixed in the same way as in Schaudinn's fluid but can be kept in the fixative for 1 hour.

Tissues can be fixed for several hours but not longer than 18 hours.

After fixation, the smears or tissues are kept in 70 per cent. alcohol (several changes) until the picric acid is extracted, when they are ready for staining.

For fixation of the insect-vectors of blood protozoa very good results can be obtained with alcoholic picro-formol to which 1-2

drops of chloroform are added. During the first hour the receptacle containing the solution is kept warm (e.g. on the top of a paraffin oven), after which fixation is continued at room temperature overnight (total time up to 18 hours).

## 2. Staining

Of the numerous methods of staining protozoa the most satisfactory results are obtained with the hæmatoxylin stains. A number of these are described below, the choice depending on whether or not a rapid diagnosis is required, and on the type of protozoa dealt with.

Before proceeding with the staining, the preparations—both smears and sections—are transferred from 70 per cent. alcohol, in which they have been kept after fixation, to distilled water for about 5 minutes.

### HEIDENHAIN'S IRON HÆMATOXYLIN

#### Composition

A 0·5 per cent. solution is made by dissolving 1 g. hæmatoxylin in 10 ml. absolute alcohol with the aid of heat, and adding 190 ml. of distilled water. The mixture is put in a stoppered bottle and kept in a warm place, exposed to light (preferably sunlight), for about 2 weeks, when it ripens, i.e. the hæmatoxylin is partially oxidized to hæmatein, and is ready for use.

A 4 per cent. aqueous solution of iron alum.

#### Procedure

Smear-preparations or sections are dealt with as follows:—

- i. Mordant in 4 per cent. iron alum for 6 hours,
- ii. Wash in distilled water,
- iii. Stain in hæmatoxylin for 6 hours or overnight.

Iron hæmatoxylin overstains the parasites and other elements present, and the preparation, when first stained, is black. In order to produce the desired effect, the preparation must be differentiated to extract the excess of stain, as follows: the preparation is rinsed in distilled water and transferred to 1–2 per cent. iron alum in which it is kept for a few minutes, then rinsed again and examined under the microscope, the process being repeated until the staining is

satisfactory. Differentiation under microscopic control is a laborious process requiring some experience. When the organisms are scanty or their presence has not been definitely established, it is best to stain four smears and to differentiate the preparations without microscopic control, by removing one at a time after 1, 2, 3, and 4 minutes. In this way at least one of the preparations might reveal well-stained protozoa. Care should be taken not to over-extract the stain, otherwise it may be necessary to re-stain the preparation. Microscopic examination of the wet preparation is considerably facilitated if a water-immersion objective is available. If not, it can be examined under medium power ( $\frac{1}{8}$  in. objective and a high power ocular).

After differentiation, the preparation is "blued" by washing in running tap-water for half an hour. It is then dehydrated by passing through ascending strengths of alcohol—70 per cent., 90 per cent. and absolute alcohol—leaving it 5–10 minutes in each; lastly, it is cleared in xylol and mounted in Canada balsam.

While iron hæmatoxylin is one of the best cytological stains for all kinds of protozoa, it is also the most difficult to use, and is, therefore, not recommended for purely routine purposes.

### MAYER'S ACID HÆMALUM (Modification)

#### Composition

1 g. hæmatoxylin is dissolved in 1 litre of distilled water; to this solution are added 0.2 g. iodate of sodium ( $\text{NaIO}_3$ ) and 50 g. potash alum; after these have dissolved, the solution is filtered and 20 ml. glacial acetic acid are added to it.

#### Procedure

Smear-preparations and sections can be stained with hæmalum for from 20 to 60 minutes. Since it does not overstain, no differentiation is required. The subsequent proceedings are the same as with Heidenhain's hæmatoxylin ("bluing" in running tap-water, dehydration, clearing and mounting in balsam).

This stain is most suitable for sections of tissues containing protozoa, but smears with intestinal protozoa do not stain satisfactorily with hæmalum. However, it can be employed for these parasites if rapid diagnosis is required.

## DOBELL'S TUNGSTIC HÆMATOXYLIN

## Composition

0.5 per cent. solution of hæmatoxylin, made and ripened as described for Heidenhain's iron hæmatoxylin, and 2 per cent. aqueous solution of phosphotungstic acid.

## Procedure

(i) Mordant the preparation in 2 per cent. phosphotungstic acid for 1-3 hours.

The actual staining can be carried out in two ways : by Dobell's original method or by Cooper's modification of this method.

## DOBELL'S METHOD

(iia) Wash in several changes of distilled water, then stain in hæmatoxylin  $\frac{1}{2}$  to 3 hours. (The longer time is required for cysts and flagellates, the shorter for active amœbæ.)

## COOPER'S METHOD

(iib) Without washing, transfer directly to hæmatoxylin and stain overnight in incubator at 37° C.

(iii) The subsequent treatment is the same as in Heidenhain's and Mayer's methods, viz. the preparation is "blued" in tap-water, dehydrated, cleared and mounted.

Tungstic hæmatoxylin is suitable both for smears and for sections of material containing intestinal protozoa. While excellent preparations can be obtained by both variants, Cooper's method is more reliable and produces uniformly good results, whereas with Dobell's method the results are not always satisfactory. Therefore, Dobell's technique is recommended for rapid diagnosis and is superior in this respect to Mayer's hæmalum, while Cooper's is advisable when speed is not essential.

## MALLORY'S PHOSPHOTUNGSTIC HÆMATOXYLIN

## Composition

Hæmatoxylin	.	.	.	.	.	0.1 g.
Distilled water	.	.	.	.	.	80 ml.
10 per cent. Phosphotungstic acid	.	.	.	.	.	20 ml.
0.25 per cent. Potassium permanganate	.	.	.	.	.	7 ml.



Dissolve the hæmatoxylin, add first the acid, then potassium permanganate.

### Procedure

Preparations of intestinal flagellates are stained for 12-24 hours (overnight is convenient), while those containing amœbæ or their cysts can be stained for 1-6 hours; the preparation is then transferred *directly* to 95 per cent. alcohol for 5-10 minutes, after which it is rapidly dehydrated in absolute alcohol, cleared in xylol and mounted.

Mallory's method is one of the best stains for flagellates, while the staining of amœbæ and their cysts is quite satisfactory for diagnostic purposes.

### Counterstain

The appearance of the protozoa stained with hæmatoxylin can be improved by counterstaining with 1 per cent. aqueous solution of eosin, which imparts to the cytoplasm a pink tint. It can be used with Heidenhain's, Mayer's and Dobell's stains. After removal of the preparation from tap-water, it is dipped for  $\frac{1}{2}$ -1 minute into the eosin solution, then dehydrated, cleared and mounted in the usual way.

### Choice of Methods

(a) *Intestinal Protozoa*.—For routine purposes, intestinal protozoa are examined in fresh fæcal preparations. Permanent preparations for rapid diagnosis are made by fixing fæcal smears in Schaudinn's fluid and staining with tungstic hæmatoxylin by Dobell's method or with Mayer's hæmalum. For cytological purposes, amœbæ and their cysts are stained with tungstic hæmatoxylin by Cooper's method, while flagellates are stained by Mallory's method.

(b) *Blood Protozoa*.—The routine examination of blood and tissue protozoa (malaria parasites, leishmanias, trypanosomes and toxoplasms) is made in blood films and tissue smears stained by one of the Romanovsky methods. For sections, portions of the infected tissues are fixed in Zenker's fluid or in Bouin's picro-formol, and the sections are stained with Heidenhain's hæmatoxylin or Mayer's hæmalum.

(c) Insect-Vectors.—The entire insect or parts containing chitin are fixed for sections in Dobell's alcoholic picro-formol with addition of chloroform, while soft organs, when dissected out (e.g. the alimentary canal), can be fixed in Zenker's fluid or Bouin's picro-formol without chloroform. The sections can be stained with Mayer's hæmalum or Heidenhain's hæmatoxylin, and counter-stained with eosin. Organ smears of insects infected with leishmanias or trypanosomes are treated like smears of human tissues. The appearance of these flagellates in stained preparations is sometimes improved if the moist smears are exposed to *osmic vapour* before drying (for technique see Chapter 16).

## CHAPTER 19

### CULTIVATION

CULTIVATION of parasitic protozoa in artificial media is resorted to with various ends in view. It can be used for the study of the life-cycle and physiology of the organisms, for *in vitro* tests of drugs or as a laboratory method for the diagnosis of infection. In the case of some protozoa (i.e. intestinal parasites) growth in culture imitates their development in the mammalian host: such cultures are maintained at blood temperature. Other protozoa, the life-cycle of which involves an alternation of hosts, behave in culture as they do in the invertebrate host (e.g. leishmanias and trypanosomes): such organisms are grown at a temperature at which the vector normally lives. The description of the cultivation of some of the parasitic protozoa of man, which follows, is restricted primarily to those methods which can be employed for the practical diagnosis of protozoal diseases. Needless to say, all sterile precautions should be observed in handling cultures.

#### MEDIUM FOR CULTIVATION OF HUMAN ENTAMÆBÆ

At present there is a great variety of media used for the maintenance of cultures of human entamæbæ, their suitability depending on a number of factors, especially the accompanying intestinal flora. The most reliable medium for the primary isolation and maintenance of *Entamæba histolytica* is Dobell's "HSre," which is prepared as follows:

#### DOBELL'S "HSre" MEDIUM

(1) Rice starch is placed in a flat dish and dried in an incubator, then distributed in small tubes (about 2.5 g. in each), and finally sterilized by dry heat at 180° C. for 1 hour.

(2) Ringer's solution (modified formula) is made up: sodium chloride 9 g.; potassium chloride 0.2 g.; calcium chloride 0.2 g.; distilled water 1 litre.

(3) Liquid part of the medium (covering fluid) is prepared as follows:—

- (a) Whole eggs (4) are scrubbed with soap and water and then disinfected in Dakin's solution (25 g. sodium bicarbonate + 720 ml. "Milton" made up to 4 litres with distilled water) for 10 minutes, after which they are flushed with alcohol, which is flamed off;
- (b) A window is cut out of the blunt end of the egg-shell with a pair of sterile scissors; the egg is inverted over a flask containing 1 litre of Ringer's solution (see 2), then the opposite (pointed) end of the egg is punctured with the scissors, allowing the white to run into the flask;
- (c) The egg albumin and Ringer's solution are thoroughly mixed and the mixture is sterilized through a filter (large Berkefeldt N or Seitz);

(4) Solid part of the medium (slant) is prepared as follows:—

- (a) Whole inactivated horse serum (sterilized by filtration) is poured out into test-tubes (about  $\frac{1}{3}$  full) with aseptic precautions;
- (b) The tubes are placed in an inspissator at an angle, to produce slants (about  $1\frac{1}{2}$  in. in length and not reaching the bottom of the test-tube), and are heated to  $80^{\circ}\text{C}$ . for 1 hour 10 minutes, to coagulate the serum;
- (c) The test-tubes are removed and incubated to test for sterility.

(5) The slants are covered with the sterile covering fluid (Ringer-egg albumin) sufficiently to reach the top of the serum slope;

(6) The completed tubes are again incubated to test sterility;

(7) Rice starch may be added immediately before use (platinum loop is first dipped in the covering fluid and then into starch, and a loopful of this is dropped into the fluid part of the medium).

#### Method of Cultivation of *E. histolytica*

Cultures do not represent a reliable diagnostic method for the detection of *E. histolytica*, since in some cases the faecal flora is not suitable for their growth. However, cultures may serve as a useful supplementary method, confirming the results of the microscopic examination of the stool.

Since the initial culture sometimes grows better if acriflavine is added to "HSre" medium, it is advisable to inoculate two

tubes: one with, the other without acriflavine (for amount see below).

To start a culture, a large platinum loopful of fæces (size of small pea), containing the amœbæ or their cysts, is introduced into the tube, at the base of the slope, and incubated at 37° C.

Newly isolated cultures should be examined daily for several days. If growth of amœbæ is observed, the culture can be sub-inoculated into fresh medium, after which it is maintained by subcultures made every 2-4 days.

The amœbæ in the culture are recovered from the surface of the starch at the base of the slant by means of a sterile pipette with a teat attached to it, care being taken not to introduce air-bubbles and not to disturb the deposit unduly.

A portion of the sample taken up in the pipette is examined microscopically on a slide, under a coverslip: the amœbæ are clearly visible with  $\frac{3}{4}$  in. objective and  $\times 10$  or  $\times 15$  ocular, or with  $\frac{1}{2}$  in. objective and  $\times 5$  (= No. 2) ocular.

Sometimes difficulty is experienced in establishing a culture, and growth of amœbæ is poor, owing to rapid multiplication of starch-splitting microorganisms. Such cultures can be "purified" by acriflavine, a 1:20,000 dilution of which kills the above-named organisms, as well as some others, but has no effect upon *Bacillus* (= *Escherichia*) *coli*, which is capable of supporting growth of *E. histolytica*. The latter is not killed by a 1:10,000 dilution of flavine. The development of this amœba may also be inhibited by the growth in culture of a unicellular fungus, *Blastocystis hominis*, which is commonly present in the human intestine (see Chapter 16). However, this fungus does not thrive in the presence of starch, therefore the elimination of starch-splitting microorganisms also serves--by preserving the starch--to inhibit excessive growth of the fungus.

"Purification" of the culture is carried out as follows: prepare 1:500 solution of acriflavine in saline; to each tube, containing about 5 ml. covering fluid, add 0.2-0.25 ml. of the diluted flavine, to give 1:12,000-1:10,000 dilution of the chemical in the medium. A large loopful of starch is then added to the tube and the amœbæ are inoculated into this medium. If satisfactory growth is obtained in the acriflavine-treated medium, the next subculture is made into ordinary medium. If growth in this is still poor, several sub-

cultures into acriflavine-treated medium might be required. As stated above, this method can also be used for isolating the primary culture from the stool.

### MEDIA FOR CULTIVATION OF HÆMOFLAGELLATES

Cultivation of hæmosflagellates (leishmanias and trypanosomes) can only be effected if the infective material (blood or tissues) is taken under strictly aseptic conditions, as the presence of extraneous microorganisms inhibits the growth of these flagellates.

In all hæmosflagellates the forms which appear in culture are of the same type as their stages of development in the insect-vector. Cultures thus imitate their life-cycle in the invertebrate host. This peculiarity determines the temperature at which the cultures will grow: this is considerably below 37° C. and approaches the temperature at which the vector normally lives.

The cultural method is of real diagnostic value only in leishmaniasis and in Chagas' disease, whereas its application in Sleeping Sickness is of less practical importance.

The media employed are as follows:—

#### "N.N.N." MEDIUM

Novy-MacNeal-Nicolle's medium (N.N.N.) is employed for the cultivation of *Leishmania* and *Trypanosoma cruzi*, and is also suitable for the non-pathogenic trypanosomes of the *lewisii*-group, but not for the African human trypanosomes (*T. gambiense* and *T. rhodesiense*).

#### Preparation

- (a) 225 ml. tap-water are measured out into 500 ml. flask; 3.5 g. agar-agar powder and 1.5 g. sodium chloride are weighed out and added to water in flask, and thoroughly shaken;
- (b) The mixture is steamed 2 hours;
- (c) Filtered through cotton wool into clean flask;
- (d) Dispensed into sterile test-tubes (with cotton wool plugs) in 5 ml. amounts (about 1 in. up the tube);
- (e) The tubes and contents are autoclaved for 20 minutes at 120° C.;
- (f) The tubes are removed to water-bath maintained at 45°–50° C.;

- (g) 20 drops whole rabbit blood are added to each tube under strictly aseptic conditions ;
- (h) The contents are mixed by rolling the tubes between palms of the hands, being careful to avoid formation of air bubbles ;
- (i) The tubes are allowed to set in sloping position without leaving a butt of medium at base of tube ;
- (j) The tubes are capped with sterile rubber caps or with cotton wool plugs sealed with paraffin wax ;
- (k) They are incubated at 37° C. for 24 hours, to test for sterility and to express water of condensation ; and
- (l) Stored in cool dark cupboard.

### Procedure

The infective material is inoculated into and grown in the condensation fluid at the bottom of the tubes, at 22-24° C. Subcultures are made once a week or fortnightly.

### ADLER'S MEDIUM (Modification)

This medium is used for the cultivation of *Leishmania*.

### Preparation

(a) LEMCO-AGAR: laboratory Lemco 5 g., agar (powder) 25 g., peptone 10 g., sodium chloride 5 g., distilled water 1 litre.

(i) Above solution is steamed for 2 hours ; (ii) cooled to 56° C. and pH set to 7.4 ; (iii) whites of 2 eggs are added ; (iv) steamed 1 hour (to coagulate) ; (v) filtered through Chardin paper in steam ; (vi) placed in bottle, and (vii) autoclaved at 15 lbs. for 15 minutes.

(b) ADLER'S MEDIUM: to 160 ml. of Ringer's solution (NaCl 9.0 g., KCl 0.42 g., CaCl<sub>2</sub> 0.24 g., NaHCO<sub>3</sub> 0.2 g., distilled water 1 litre) are added 0.3 g. of glucose and 20 ml. of Lemco-agar (a) ; the solution is then (i) distributed in tubes, 3-4 ml. in each ; (ii) autoclaved at 15 lbs. for 15 minutes ; (iii) to each tube 5 drops of normal rabbit or horse blood are added aseptically ; (iv) the tubes are incubated at 37° C. for 24 hours, to test for sterility.

### Procedure

The material with leishmanias is inoculated on to the surface of the medium, and the cultures are kept at 22° C., subcultures being made once a week or fortnightly.

## YORKE'S MEDIUM

For the study *in vitro* of the effect of drugs upon trypanosomes (*Trypanosoma gambiense* and *T. rhodesiense*) and of their metabolism, as well as for temporary preservation, a medium is used in which the trypanosomes can be kept alive for 24 hours at 37° C., while retaining their blood form.

## Preparation

## (a) RINGER-GLUCOSE SOLUTION

Sodium chloride . . . . .	0.9 g.
Potassium chloride . . . . .	0.025 g.
Calcium chloride . . . . .	0.020 g.
Sodium bicarbonate . . . . .	0.015 g.
Glucose . . . . .	0.2 g.
Distilled water . . . . .	100 ml.

## (b) CITRATE-RINGER-GLUCOSE SOLUTION

This is made up by adding to the Ringer-glucose solution (a) sodium citrate to give a 1 per cent. solution.

## (c) YORKE'S MEDIUM

This is made up by mixing equal parts of rabbit serum and Ringer-glucose solution (a).

## Procedure

Some of the blood containing trypanosomes is added to a centrifuge tube filled with citrate-Ringer-glucose solution (b) and centrifuged at low speed for 3 minutes. With a pipette, the trypanosomes, together with a drop of supernatant fluid, are removed from the top of the deposit and put into a tube containing Yorke's medium (c).

In a sample of this suspension the number of trypanosomes is counted with a hæmocytometer. Their density should not exceed 1,000 per 1  $\mu$ l. (c.mm.) (about 6 trypanosomes per large square of the counting chamber), otherwise they die rapidly, owing to consumption of the glucose in the medium. The required concentration can be obtained by further dilution with the fluid (c). At the right concentration the trypanosomes remain viable, in undiminished numbers, at the end of 24 hours, when kept in an incubator at 37° C.



- (g) 20 drops whole rabbit blood are added to each tube under strictly aseptic conditions ;
- (h) The contents are mixed by rolling the tubes between palms of the hands, being careful to avoid formation of air bubbles ;
- (i) The tubes are allowed to set in sloping position without leaving a butt of medium at base of tube ;
- (j) The tubes are capped with sterile rubber caps or with cotton wool plugs sealed with paraffin wax ;
- (k) They are incubated at 37° C. for 24 hours, to test for sterility and to express water of condensation ; and
- (l) Stored in cool dark cupboard.

### Procedure

The infective material is inoculated into and grown in the condensation fluid at the bottom of the tubes, at 22-24° C. Subcultures are made once a week or fortnightly.

### ADLER'S MEDIUM (Modification)

This medium is used for the cultivation of *Leishmania*.

### Preparation

(a) LEMCO-AGAR: laboratory Lemco 5 g., agar (powder) 25 g., peptone 10 g., sodium chloride 5 g., distilled water 1 litre.

(i) Above solution is steamed for 2 hours ; (ii) cooled to 56° C. and pH set to 7.4 ; (iii) whites of 2 eggs are added ; (iv) steamed 1 hour (to coagulate) ; (v) filtered through Chardin paper in steam ; (vi) placed in bottle, and (vii) autoclaved at 15 lbs. for 15 minutes.

(b) ADLER'S MEDIUM: to 160 ml. of Ringer's solution (NaCl 9.0 g., KCl 0.42 g., CaCl<sub>2</sub> 0.24 g., NaHCO<sub>3</sub> 0.2 g., distilled water 1 litre) are added 0.3 g. of glucose and 20 ml. of Lemco-agar (a) ; the solution is then (i) distributed in tubes, 3-4 ml. in each ; (ii) autoclaved at 15 lbs. for 15 minutes ; (iii) to each tube 5 drops of normal rabbit or horse blood are added aseptically ; (iv) the tubes are incubated at 37° C. for 24 hours, to test for sterility.

### Procedure

The material with leishmanias is inoculated on to the surface of the medium, and the cultures are kept at 22° C., subcultures being made once a week or fortnightly.

This method is also used for *in vitro* observation of the development of the coccidia parasitic in other animals, the identification of which is based on the characters of the mature oocyst. The oocysts can be kept in the acid solution for long periods of time (in some cases up to one year) without losing their viability and infectivity.

## RAZGHA'S MEDIUM

(BRUTSAERT and HENRARD's modification)

This medium is used for the cultivation of the pathogenic human trypanosomes (*T. gambiense* and *T. rhodesiense*), which will not grow in any of the media used for non-pathogenic trypanosomes and leishmanias.

## Preparation

(a) Into each of the desired number of test-tubes are poured 2-2.5 ml. of Ringer's solution containing 0.6 per cent. sodium chloride, or the same volume of Tyrode's fluid, and the tubes are autoclaved ;

(b) To each of these tubes are added 2 ml. normal human or rabbit blood containing 1 per cent. sodium citrate ; the tubes are incubated to test for sterility and kept in the cold ;

(c) 1 ml. ampoules are filled with 1 per cent. solution of "Liquoïde Roche" (= sodium polyanethyl sulphonate) ; these are sealed, autoclaved and will keep indefinitely.

## Procedure

To obtain blood for culture 1 ml. of "Liquoïde Roche" (c) is taken into a syringe, then 5 ml. of blood are drawn into it from a vein (basilic). The contents of the syringe are mixed and 0.5 ml. of the blood mixture is put into a test tube containing (a) + (b). The tube is incubated at 25-28° C. for 10 days. Subcultures are made every 10 days.

MAINTENANCE OF OOCYSTS OF COCCIDIA *IN VITRO*

Oocysts of the human *Isospora*, when voided in the stool, contain an unsegmented zygote, the further development of which (sporogony) can be observed *in vitro*. Faeces with the oocysts are spread out in a Petri dish containing 0.5 or 1 per cent. solution of chromic acid, or 2 per cent. potassium bichromate, and covered with a lid. The acid inhibits the growth of fungi and bacteria, while the development of the coccidium is unimpaired and proceeds at room temperature, being completed in from 1 to 4 days. It can be observed by periodical examination of a sample of the faeces until mature oocysts, containing two sporocysts, each with four sporozoites, are produced.

This method is also used for *in vitro* observation of the development of the coccidia parasitic in other animals, the identification of which is based on the characters of the mature oocyst. The oocysts can be kept in the acid solution for long periods of time (in some cases up to one year) without losing their viability and infectivity.

## DETECTION OF PROTOZOA IN INSECT-VECTORS

THE insect-vectors of protozoal diseases of the blood and reticulo-endothelial system are examined with two main objects in view: (a) for the study of the stages of development of the parasite in the intermediate host, and (b) for the determination of the presence or absence of infection with such parasites (e.g. in surveys, transmission experiments or xenodiagnosis). The first object might necessitate fixation of the entire insect, or of separate parts of it, for histological sections, and has already been dealt with (Chapter 18), whereas the second object—with which we are primarily concerned here—can be attained by dissection of the insect and the removal of the organs in which the developmental stages of the parasite are to be found, while in some cases the operation can be further simplified, with considerable saving of time.

The instruments required for dissection are fine scissors, forceps, dissecting needles and a dissecting microscope (mono- or binocular).

## i. MOSQUITOS (Figs. 37, 42)

Mosquitos are dissected for the detection of the oocysts of malaria parasites on the stomach wall and of sporozoites in the salivary glands. The insect can be killed by concussion (shaking the test-tube in which it is placed). For dissection, the wings and legs are plucked off with the fingers and the mosquito is laid down on its side in a drop of saline on a slide.

To remove the salivary glands, the thorax is held down with a needle in the left hand, while a needle in the right hand is placed behind the head which is gently pulled away from the thorax. With practice the head usually comes off together with the salivary glands, which can be recognized as two refractile three-lobed tubules. The salivary glands are placed in a small drop of saline, between slide and coverslip, which is pressed to release the sporozoites from the glands. The sporozoites can now be examined in the fresh preparation. Permanent preparations can be made by teasing up the glands and making a smear on a slide, drying it in the air and staining by one of the Romanovsky methods.

To remove the stomach, the thorax is held down with a needle in the left hand, a nick is made through the chitin on the dorsal and ventral surfaces of the tip of the abdomen, then a needle is applied to the extreme end of the abdomen and the gut, including the stomach, is gradually dragged out. The stomach is then freed of

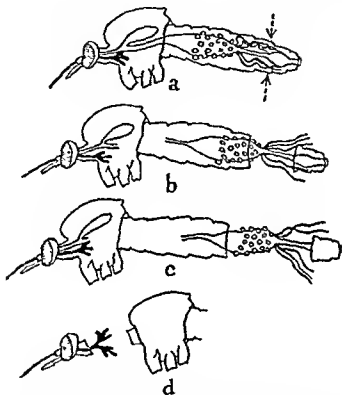


FIG. 42.—DISSECTION OF MOSQUITO FOR MALARIA PARASITES.  
(Adapted from Brumpt, 1936.)

a. Arrows indicate points at which incisions are made in the chitinous covering of the abdomen; b, c. Alimentary tract, including stomach with oocysts on its wall, being drawn out; d. Separation of head from thorax and extraction of salivary glands.

the surrounding viscera, placed in a drop of saline between slide and coverslip, and examined microscopically for oocysts on its surface. A permanent preparation of the entire stomach with oocysts can be made by placing a drop of Schaudinn's fluid at the edge of the coverslip and drawing a corresponding amount of saline from the opposite side, and repeating the process until the fixative has reached all parts of the stomach, after which a larger quantity of fixative

is allowed to run in, raising the coverslip. Fixation is continued for another 10-15 minutes, after which the flattened stomach is dealt with in the usual manner and stained with Mayer's acid hæmalum.

## ii. SANDFLIES (Figs. 23, 24)

The developmental stages of *Leishmania*, represented by leptomonad flagellates (Fig. 22), are found in the alimentary tract of the sandfly. In view of the minute size of these insects, it is advisable to use a binocular microscope and fine needles, which can be made by mounting an entomological pin in a wooden holder. The fly is placed on a slide in a drop of saline and all the body hairs are brushed away with a fine camel hair brush, while the legs are removed with a needle. The fly is then placed on its side in a small drop of fluid (saline, 4 per cent. glucose or dilute serum). One needle is placed on the thorax, another on the head, which is pulled out with the œsophagus. The rest of the gut can be withdrawn with a needle applied to the posterior end of the abdomen.

Alternatively, the head is first cut off, then, with a needle at the posterior end of the abdomen, the entire gut can be extracted. The flagellates can be detected by teasing up the gut in a droplet of fluid. For permanent preparations it is advisable to use 4 per cent. glucose or dilute serum, the teased-up contents of the gut being smeared on a slide and treated like a thin blood film (see Chapter 18). For demonstrating the flagellates *in situ* infected sandflies are fixed and examined in sections.

## iii. TSETSE FLIES (Figs. 26, 27, 43)

The stages of development of various African trypanosomes may be found in the mouth-parts, salivary glands and alimentary tract of *Glossina* (Fig. 27). Before dissection, the tsetse fly is chloroformed; the wings and legs are removed with fine scissors, the tip of the abdomen (last two segments) is cut off, and the fly is placed on its back on a slide with a drop of fluid (saline, dilute serum or 4 per cent. glucose solution) in contact with the cut end. The body is held with the fingers of the left hand, while a dissecting needle held in the right hand is placed across the anterior end of the abdomen and drawn, with slight pressure, towards the posterior end, until the viscera are gradually forced out on to the slide. By

this method almost the entire intestine, from proventriculus to rectum, together with the crop and salivary glands, can be extracted. If the glands have been left behind, they can be pressed out by repeating the process described above, but the fly should be placed in a watch glass with more fluid. However, in some cases it may be necessary to dissect out the glands and their ducts, after making a longitudinal incision through the thorax and abdomen somewhat laterally to the median line. After removal of the viscera the slide is placed under a dissecting microscope (mono- or binocular) and the alimentary canal and salivary glands are gradually separated from the other tissues, and placed in separate drops of fluid.

For the detection of trypanosomes, the gut is cut into portions and the contents expressed and placed between slide and coverslip, while the salivary glands can be mounted totally under a coverslip. Microscopic examination is carried out with a  $\frac{1}{8}$  in. objective.

The salivary glands can also be removed independently in the following manner: the fly, placed in a drop of fluid on a slide, is held in the fingers of the left hand, while a needle, held in the right hand, is placed across the "neck" (junction of head and thorax) and manipulated gently until the chitin is severed, after which traction is exerted on the head until the salivary glands are withdrawn from the body.

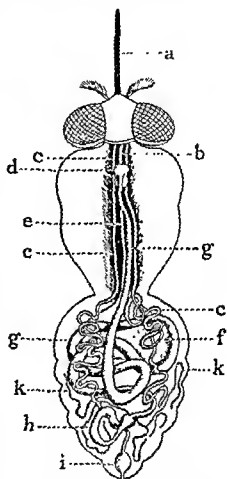


FIG. 43.—ALIMENTARY TRACT AND ACCESSORY ORGANS OF TSETSE-FLY (*Glossina*) *in situ*. (Adapted from various authors.)

a. Proboscis; b. Oesophagus; c. Salivary glands; d. Proventriculus; e. Duct of crop (f); g. Midgut; h. Hindgut with rectum (i); k. Malpighian tubes.



For examination of the proboscis, its constituents are separated as follows: first the labium is drawn away from the labrum and its bulb by placing the points of two needles on the bases of these parts. The hypopharynx may remain in the groove of the labium or within the labrum, from which it is removed by pressing on the bulb with one needle, while with the other the hypopharynx is drawn out. The whole labrum with its bulb is then cut off. The isolated labium and hypopharynx are examined microscopically for trypanosomes under a coverslip in one of the above-named fluids. The procedure can be simplified as follows: the fly is first held in the right hand, and the palps are bent over to the dorsal surface, after which the fly is lifted by the proboscis held with two fingers of the left hand, the proboscis is severed behind the bulb and placed in a drop of fluid on a slide for microscopic examination. If necessary the different parts of the proboscis can be separated by exerting slight pressure on the coverslip.

For permanent preparations of the trypanosomes found in any of the organs dissected out, portions of these are teased up in a droplet of 4 per cent. glucose or dilute serum, smeared on a slide, dried in the air and stained by one of the Romanovsky methods. Saline should not be used for this purpose, as it may interfere with the staining of the trypanosomes.

In experiments on the transmission of trypanosomes and in surveys, it may be necessary to detect and isolate tsetse flies having a salivary gland infection, i.e. those in which the trypanosomes have completed their cycle of development and which are infective. It might also be desirable to count the number of trypanosomes ejected by the fly when it feeds. The best results are obtained with Burt's technique, which is as follows: (a) a microscopic slide is lightly smeared with egg albumin and allowed to dry; (b) a hungry fly is placed in a small bottle, having a mouth about  $1\frac{1}{4}$  in. wide and closed with mosquito netting; (c) the bottle with the fly is clamped, mouth downwards, in a burette-stand, and a guineapig is placed close to the netting in order to stimulate probing; (d) as the fly extrudes its proboscis through the netting in an attempt to feed, a prepared slide is interposed so as to come in contact with the tip of the proboscis, with the result that the fly probes on the slide, ejecting some of the saliva which may contain trypanosomes. When the fly retracts its proboscis, the slide is dried in the air and

stained like a blood film by one of the Romanovsky methods. If metacyclic trypanosomes are absent, probing can be repeated over a period of 10 days, whenever the fly is hungry.

#### iv. REDUVIID BUGS (Figs. 30, 31)

The developmental stages of *Trypanosoma cruzi* (Fig. 28) in Reduviid bugs are found in the gut exclusively. The bug is laid, with the dorsal surface upwards, on a slab of paraffin wax or on a piece of cork, and fixed in position with four pins arranged crosswise. With fine scissors a cut is made around the lateral margins of the abdomen and thorax, the dorsal walls of which are lifted with fine forceps, thus exposing the viscera. These are moistened with a drop of fluid (saline, 4 per cent. glucose or dilute serum), the gut is dissected out, transferred to another drop of fluid on a slide and its contents are examined for flagellates under the microscope.

For xenodiagnosis the results can be assessed merely by examination of the faeces for the presence of flagellates. This can be done either by placing a recently fed bug in a specimen tube and collecting its droppings, or by provoking defecation as follows: the bug is held between thumb and index of the left hand, while the point of a fine entomological pin (preferably mounted in a handle) is gently introduced into the anal aperture and then withdrawn. The anus is then brought in contact with a drop of one of the above fluids and a small amount of faeces is expelled by pressure on the abdomen. The drop is then mounted under a coverslip and examined microscopically.

For permanent preparations the technique is the same as described for trypanosomes recovered from tsetse flies.

100



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